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APPLICATION

FOR

UNITED STATES LETTERS PATENT

APPLICANT : DENNIS GONSALVES and BAOZHONG MENG

TITLE : RUPESTRIS STEM PITTING ASSOCIATED VIRUS NUCLEIC ACIDS, AND THERE USES

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RUPESTRIS STEM PITTING PITTING ASSOCIATED VIRUS NUCLEIC ACIDS, PROTEINS, AND THEIR USES

This application claims the benefit of U.S. Provisional Patent Applications Serial Nos. 60/047,147, filed May 20, 1997, and 60/069,902, filed December 17, 1997. This work was supported by the U.S. Department of Agriculture Clonal Repository – Geneva, Grant Nos. 58-2349-9-01 and 58-2349-9 and U.S. Department of Agriculture Cooperative Agreement Grant Nos. 58-1908-4-023, 58-3615-5-036, and 58-3615-7-060. The U.S. Government may have certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates to *Rupestris* stem pitting associated virus ("RSPaV") proteins, DNA molecules encoding these proteins, and diagnostic and other uses thereof.

BACKGROUND OF THE INVENTION

The world's most widely grown fruit crop, the grape (*Vitis sp.*), is cultivated on all continents except Antarctica. However, major grape production centers are in European countries (including Italy, Spain, and France), which constitute about 70% of the world grape production (Mullins et al., Biology of the Grapevine, Cambridge, U.K.:University Press (1992)). The United States, with 300,000 hectares of grapevines, is the eighth largest grape grower in the world. Although grapes have many uses, a major portion of grape production (~80%) is used for wine production. Unlike cereal crops, most of the world's vineyards are planted with traditional grapevine cultivars, which have been perpetuated for centuries by vegetative propagation. Several important grapevine virus and virus-like diseases, such as grapevine leafroll, corky bark, and *Rupestris* stem pitting ("RSP"), are transmitted and spread through the use of infected vegetatively propagated materials. Thus, propagation of certified, virus-free materials is one of the most important disease control measures. Traditional breeding for disease resistance is difficult due

to the highly heterozygous nature and outcrossing behavior of grapevines, and due to polygenic patterns of inheritance. Moreover, introduction of a new cultivar may be prohibited by custom or law. Recent biotechnology developments have made possible the introduction of special traits, such as disease resistance, into an established cultivar without altering its horticultural characteristics.

Many plant pathogens, such as fungi, bacteria, phytoplasmas, viruses, and nematodes can infect grapes, and the resultant diseases can cause substantial losses in production (Pearson et al., Compendium of Grape Diseases, American Phytopathological Society Press (1988)). Among these, viral diseases constitute a major hindrance to profitable growing of grapevines. About 34 viruses have been isolated and characterized from grapevines. The major virus diseases are grouped into: (1) the grapevine degeneration caused by the fanleaf nepovirus, other European nepoviruses, and American nepoviruses, (2) the leafroll complex, and (3) the rugose wood complex (Martelli, ed., Graft Transmissible Diseases of Grapevines, Handbook for Detection and Diagnosis, FAO, UN, Rome, Italy (1993)).

Rugose wood (RW) complex is a term to describe a group of graft-transmissible diseases which are important and widespread on grapevines grown world-wide. Symptoms of RW are characterized by pitting, grooving, or distortion to the woody cylinder of the grapevine scion, rootstock, or both. Based on symptoms developed on different indicator plants after graft inoculation, RW complex can be divided into four components: Kober 5BB stem grooving (KSG), LN 33 stem grooving (LNSG), grapevine corky bark (GCB), and *Rupestris* stem pitting (RSP) (Martelli, "Rugose Wood Complex," in Graft-Transmissible Diseases of Grapevines, Handbook for Detection and Diagnosis, pp. 45-54, Martelli, ed., Food and Agriculture Organization of the United Nations, Rome, Italy (1993)). Because RW can cause severe decline and death to grapevines (Savino et al., "Rugose Wood Complex of Grapevine: Can Grafting to *Vitis* Indicators Discriminate Between Diseases?", in Proceedings of the 9th Meetings of the International Council for the Study of Viruses and Virus Diseases of the Grapevine, Anavim, Israel (1989); Credi and Babini, "Effect of Virus and Virus-like Infections on the Growth of Grapevine Rootstocks," Adv. Hort. Sci., 10:95-98 (1996)), it has been included in healthy grapevine detection schemes used in major grapevine growing countries including Italy, France, and the United States.

RSP was discovered in California in the late 1970s (Prudencio, "M. Sc. Thesis: Comparative Effects of Corky Bark and *Rupestris* Stem Pitting Diseases on Selected Germplasm Lines of Grapes," University of California, Davis, California, 36 pages (1985); Goheen, "*Rupestris* Stem Pitting," in Compendium of Grape Diseases, p. 53, Pearson and Goheen, eds., American Phytopathological Society Press, St. Paul, Minnesota, USA (1988) ("Goheen")). The disease was defined by Goheen as follows: after graft inoculation with a chip bud from an infected grapevine, the woody cylinder of the indicator plant *Vitis rupestris* Scheele St. George ("St. George") develops a narrow strip of small pits extending from the inoculum bud to the root zone. Grafted St. George plants were checked for wood symptoms 2 to 3 years after inoculation. In contrast to GCB, which elicits pitting and grooving on St. George and LN 33, RSP does not produce symptoms on the latter (Goheen, "*Rupestris* Stem Pitting," in Compendium of Grape Diseases, p. 53, Pearson and Goheen, eds., American Phytopathological Society Press, St. Paul, Minnesota, USA (1988)).

RSP is probably the most common component of the RW complex on grapevines. Surveys in California revealed a high disease incidence in many grapevine cultivars imported from Western Europe and Australia (Goheen, "*Rupestris* Stem Pitting," in Compendium of Grape Diseases, p. 53, Pearson and Goheen, eds., American Phytopathological Society Press, St. Paul, Minnesota, USA (1988)). An examination of indexing records in California compiled over 23 years revealed RSP infection in 30.5% of 6482 grapevine selections introduced from around the world (Golino and Butler, "A Preliminary Analysis of Grapevine Indexing Records at Davis, California," in Proceedings of the 10th Meeting of the ICVG, pp. 369-72, Rumbos et al., eds., Volos, Greece (1990)). Indexing in New York State showed that 66% of 257 grapevines tested on St. George developed typical small pits below the inoculum bud or around the woody cylinder (Azzam and Gonsalves, Abstract: "Survey of Grapevine Stem-Pitting in New York and Isolation of dsRNA from a Grapevine Selection Infected with Stem Pitting," Phytopathology 78:1568 (1988)). Furthermore, several reports have indicated that RSP is the most frequently detected component of the RW complex in Italy (Borgo and Bonotto, "Rugose Wood Complex of Grapevine in Northeastern Italy: Occurrence of *Rupestris* Stem Pitting and Kober Stem Grooving," in Extended Abstracts of the 11th Meeting of the International Council for the Study of Viruses and Virus Diseases of the Grapevine (ICVG), pp. 61-62, Gugerli, ed.,

Montreux, Switzerland (1993); Credi, "Differential Indexing Trials on Grapevine Rugose Wood Syndrome," Extended Abstracts of the 11th Meeting of the International Council for the Study of Viruses and Virus Diseases of the Grapevine (ICVG), p. 63, Gugerh, P., ed., Montreux, Switzerland (1993)).

The effect of RSP on growth, yield, and grapevine quality is not well understood and, thus, subject to debate. The reason for this ambiguity is the absence of a rapid and sensitive diagnostic tool. RSP is the most difficult grapevine disease to diagnose. Serological or molecular methods are not available for diagnosing RSP. Biological indexing on St. George, as described above, has remained the only approach to diagnose RSP. Biological indexing is labor intensive, time consuming (i.e., often requiring up to about three years to obtain results), and, by its very nature, subjective. Moreover, symptoms on St. George can be variable and not exactly as those defined by Goheen. In particular, Credi, "Characterization of Grapevine Rugose Wood Sources from Italy," Plant Disease, 82:1288-92 (1997), recently showed that some RSP infected grapevines induced pitting that is restricted to below the inoculum bud, while others induced pitting around the woody cylinder of inoculated St. George. Thus, the present method of identifying the presence of RSP is not entirely adequate.

The etiology of RSP is unknown. Efforts to isolate virus particles from RSP-infected grapevines and to mechanically transfer the causal virus(es) to herbaceous host plants failed (Azzam and Gonsalves, "Detection of in Grapevines Showing Symptoms of *Rupestris* Stem Pitting Disease and the Variabilities Encountered," Plant Disease, 75:96-964 (1991)). However, a major dsRNA species of ca. 8.3 kb, accompanied by a smaller dsRNA of ca. 7.6 kb, was consistently isolated from one Pinot Gris and four Pinot Noir clones that had been indexed positive for RSP (Walter and Cameron, "Double-Stranded RNA Isolated from Grapevines Affected by *Rupestris* Stem Pitting Disease," Am. J. of Enology and Viticulture, 42:175-79 (1991)). In addition, a third dsRNA of ca. 5.5 kb was observed in three clones. Likewise, an apparently similar dsRNA species of ca. 8.0 and 6.7 kbp was isolated from dormant canes of RSP-infected grapevines collected from California, Canada, and New York (Azzam and Gonsalves, "Detection of dsRNA in Grapevines Showing Symptoms of *Rupestris* Stem Pitting Disease and the Variabilities Encountered," Plant Disease, 75:960-64 (1991)). Six of eight Californian and three of

five Canadian samples contained these two dsRNA species. However, results of New York samples were not consistent. Among eight RSP infected grapevine selections tested, only one showed these two dsRNAs. Using explants growing in tissue culture as source materials, dsRNA of ca. 359 bp was isolated from 21 of 31 grapevine cultivars, all of which were previously indexed on St. George and considered to be infected with RSP (Monette et al., "Double-Stranded RNA from *Rupestris* Stem Pitting-Affected Grapevines," Vitis, 28:137-44 (1989)).

In view of the serious risk RSP poses to vineyards and the absence of an effective treatment of it, the need to prevent this affliction continues to exist. Moreover, the absence of a rapid and accurate diagnostic assay prevents proper identification of RSP. The present invention is directed to overcoming these deficiencies in the art.

SUMMARY OF THE INVENTION

The present invention relates to an isolated protein or polypeptide corresponding to a protein or polypeptide of a RSP virus. The encoding RNA molecule or DNA molecule, in either isolated form or incorporated in an expression system, a host cell, or a transgenic *Vitis* scion or rootstock cultivar, are also disclosed.

Another aspect of the present invention relates to a method of imparting RSP virus resistance to *Vitis* scion or rootstock cultivars by transforming them with a DNA molecule encoding the protein or polypeptide corresponding to a protein or polypeptide of a RSP virus.

The present invention also relates to an antibody or binding portion thereof or probe which recognizes proteins or polypeptides of the present invention.

Still another aspect of the present invention relates to diagnostic tests which involve methods for detecting the presence of a RSP virus in a sample. The methods include the use of an antibody or binding portion of the present invention (i.e., in an immunoassay), or a nucleic acid probe obtained from a DNA molecule of the present invention (i.e., in a nucleic acid hybridization assay or gene amplification detection procedure). The antibody or binding portion thereof, or nucleic acid probe, is introduced into contact with the sample, whereby the presence of *Rupestris* stem pitting virus in the sample is detected using an assay system.

The characterization of an RSP virus is particularly desirable because it will allow for the determination of whether the virus is associated to the specific (restricted) or nonspecific (nonrestricted) pitting symptoms of RSP, or to both. Also, RSP virus resistant transgenic variants of the current commercial grape cultivars and rootstocks allows for more complete control of the virus while retaining the varietal characteristics of specifics cultivars. Furthermore, these variants permit control over RSP virus transmitted by infected scions or rootstocks. Moreover, the diagnostic tests offer significant improvement over conventional diagnostic means currently employed, namely, rapid results and greater accuracy.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a photograph of St. George indicators which comparatively display the symptoms of RSP. The St. George indicator (a) has been graft-inoculated with infected bud wood from a grapevine accession, resulting in the indicator displaying pitting below the inoculum bud, as indicated by an arrow. This RSP symptom was defined by Goheen, "*Rupestris* Stem Pitting," in Compendium of Grape Diseases, p. 53, Pearson and Goheen, eds., American Phytopathological Society Press, St. Paul, Minnesota, USA (1988), which is hereby incorporated by reference. The St. George indicator (b) was not graft-inoculated and represents a normal appearance.

Figures 2A and 2B are photographs which respectively display the results of dsRNA analysis and Northern hybridization for dsRNA. Together the photographs may be used to correlate the dsRNA analysis of Figure 2A with the Northern hybridization (for dsRNA isolated from grapevines indexed positive for *Rupestris* stem pitting (RSP)) of Figure 2B. M. *Hind* III digested lambda DNA maker: lane 1, Aminia; lane 2, Bertille Seyve 5563; lane 3, Canandaigua; lane 4, Colobel 257; lane 5, Couderc 28-112; lane 6, Freedom; lane 7, Grande Glabre; lane 8, M 344-1; lane 9, Joffre; lane 10, Ravat 34; lane 11, Seyval; lane 12, Seyve Vinard 14-287; lane 13, Verdelet; lane 14, Pinot Noir (positive control); lane 15, Verduzzo 233A (negative control for RSP as judged by indexing on St. George); lane 16, insert of clone RSP149. Arrows indicate the position of the 8.7 kb dsRNA. With respect to lane 15 of Figure 2A, the two dsRNA bands are larger or smaller than the 8.7 kb

dsRNA associated with RSP and they did not hybridize with the RSP specific probe in Northern analysis. Thus, they are not specific to RSP.

Figure 3A is an illustration which depicts the strategy for obtaining the complete nucleotide sequence of RSPaV-1. The overlapping regions of the nucleotide sequences of the sequenced clones and RT-PCR-amplified cDNA fragments are as follows: 52-375 for RSPA/RSP28; 677-1474 for RSP28/RSP3; 3673-3766 for RSP3/RSPB; 4009-4320 for RSPB/RSP94; 5377-5750 for RSP94/RSPC; 5794-6537 for RSPC/RSP95; 6579-6771 for RSPC/RSP140; and 8193-8632 for RSP140/TA5. Figure 3B is an illustration which comparatively depicts the genome structures of RSPaV-1, ASPV, PVM, and PVX. Boxes with the same patterns represent the comparable ORFS.

Figure 4A is a comparative sequence listing of amino acid sequences of region I (aa 1-372) of RSPaV-1 ORF1 with the corresponding sequences of carlavirus PVM and ASPV. The methyltransferase motif is underlined. Capital letters indicate consensus residues. Figure 4B is a comparative sequence listing of amino acid sequences of region II (aa 1354 to end) of RSPaV-1 ORF1 with the corresponding regions of ASPV and PVM carlavirus. In Figure 4B, the NTP binding motif is underlined at (A) and the GDD containing sequence is underlined at (B). In Figures 4A and 4B, capital letters indicate consensus residues, the symbol * indicates identical amino acid residues between RSPaV-1 and ASPV, and the symbol # indicates identical amino acid residues between RSPaV-1 and PMV.

Figures 5A-D are comparative sequence listings of amino acid sequences for ORF2, ORF3, ORF4, and a C-terminal part of ORF5 (CP) of RSPaV-1, respectively, with ASPV and PVM carlavirus. In Figure 5A, the NTP binding motif, located near the C terminus of ORF2, is underlined. In Figure 5D, the conserved motif (RR/QX--XFDF), located in the central region of the coat proteins and proposed to be involved in the formation of a salt bridge structure, is underlined. In each of the figures, capital letters indicate consensus residues. The symbol * indicates identical amino acid residues between RSPaV-1 and ASPV, and the symbol # indicates identical amino acid residues between RSPaV-1 and PMV. In Figure 5D, numbers which appear in parentheses and precede the sequences indicate the start points of the C-terminal portions of CPs being compared.

Figure 6A is a comparative sequence listing of DNA nucleotide sequences for the 3' untranslated region (UTR) of RSPaV-1 and ASPV. Figure 6B is a comparative sequence listing of DNA nucleotide sequences for the 3' untranslated region (UTR) of RSPaV-1 and PVM. Clustal method of MegAlign (DNASTAR) was used to generate sequence alignments. The 21 identical consecutive nucleotides between RSPaV-1 and PVM are indicated as shadowed letters.

Figures 7A-B are photographs comparing the results of RT-PCR of grapevines using RSP149 primers (Figure 7A) and Southern blot hybridization of RT-PCR amplified cDNA fragments to RSPaV-1 specific probe (Figure 7B). MMLV-RT (Promega) was used in reverse transcription. *Taq* DNA polymerase (Promega) was used in PCR. For the RT-PCR and Southern blot hybridization: lane 1, Ehrenfelser PM1 (1169-1A1); lane 2, Cabernet franc 147A; lane 3, Chardonnay 80A; lane 4, Refosco 181A; lane 5, Touriga francesa 313; lane 6, 3309C (330-4A1); lane 7, 420A (1483-4A1); lane 8, Chardonnay 83A; lane 9, Malsavia 153A; lane 10, Aragnonex 350; lane 11, Aminia; lane 12, Chardonnay 127; lane 13, Kober 5BB 100; lane 14, Verduzzo 233A; lane 15, *V. riparia*; lane 16, *V. monticola*; lane 17, H_2O.

Figure 8 is a schematic representation of the identical genome organization among RSPaV-1 (the type strain), RSP47-4, and RSP158. The number of amino acid residues of the comparable ORFs (boxes shaded with the same pattern) among these three strains are the same (note: ORF1 and ORF5 of RSP47-4 and RSP158 are incomplete). The comparable ORFs also have high nucleotide and amino acid sequence identities, which are indicated on the bottom. Only the C-terminal portion of the ORF1 of RSPaV-1 is shown in this diagram.

Figure 9 is a comparative alignment of nucleotide sequences of seven other clones with the comparable region of RSPaV-1. Shaded areas indicate identical nucleotide sequences, whereas white boxes represent different nucleotide sequences.

Figure 10 is a schematic representation of a plant transformation vector containing the RSPaV-1 coat protein gene. This vector is designated pGA482G/RSPaV-1CP, which has the double CaMV 35S enhancers, the 35S promoter, the leader sequence of AlMV, and the 35S terminator sequence. RB, right border; LB, left border; Tet, tetracycline resistance gene; and Gent, gentamycin resistance gene.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to isolated DNA molecules encoding for the proteins or polypeptides of a *Rupestris* stem pitting associated virus. Since the nucleotide sequence was derived from cDNA clones of the dsRNA that was associated with RSP, the viral agent has been designated as *Rupestris* stem pitting associated virus ("RSPaV"). RSP is likely caused by one or a number of viral strains. The genome of each RSPaV has a plurality of open reading frames, each containing DNA molecules in accordance with the present invention. The complete genome of one strain has been sequenced and the strain is designated RSPaV-1. Substantial portions of the genomes of two other RSPaV strains have also been sequenced. These strains are designated by their clone names, RSP47-4 and RSP158.

The DNA molecule which constitutes the complete RSPaV-1 genome comprises the nucleotide sequence corresponding to SEQ. ID. No. 1 as follows:

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CGATAAACAT AACAACAGAA TCTGCATTGC AGTAATATTC CTTGAATATA ATTGCAACGC     60
AATGGCCCTC TCTTATAGGC CTGCTGTTGA AGAGGTGCTC GCAAAATTCA CCTCTGATGA    120
ACAATCCAGG GTTTCTGCTA CAGCTCTCAA GGCATTAGTA GACTTAGAGG AAAGTCAGCA    180
CAATTTGTTC TCTTTCGCAT TGCCTGATAG AAGCAAAGAA AGGCTGATAT CTTCTGGCAT    240
TTACTTAAGT CCTTACAGTT TCAGACCCCA CTCACATCCA GTTTGTAAAA CTTTAGAAAA    300
TCACATTTTG TACAATGTTT TACCTAGTTA TGTTAATAAT TCATTTTACT TTGTAGGAAT    360
CAAGGATTTT AAGCTGCAGT TCTTGAAAAG GAGGAATAAG GATCTCAGCT TGGTAGCACT    420
CATAAATAGG TTTGTGACAA GTCGTGATGT TAGTAGGTAT GGGTCTGAGT TCGTTATAAG    480
TTCTAGTGAC AAATCAAGTC AGGTTGTCAG TAGAAAGGGC ATTGGTGATT CTAACACACT    540
CCGGAGATTG GTCCCACGTG TAATTTCCAC AGGTGCCAGG AATCTTTTTC TGCATGATGA    600
GATTCACTAC TGGTCAATTA GTGATCTGAT CAATTTTTTG GACGTTGCCA AGCCAAGCAT    660
GCTCTTGGCA ACTGCAGTAA TCCCTCCAGA AGTGCTGGTT GGCTCTCCAG AGAGTCTTAA    720
CCCTTGGGCC TACCAGTATA AAATCAATGG CAACCAACTG CTCTTCGCAC CAGATGGCAA    780
CTGGAATGAG ATGTACTCAC AACCTTTGTC ATGCAGATAC CTGCTCAAGG CCAGATCTGT    840
AGTTCTGCCC GATGGCTCAC GCTACTCGGT TGACATCATT CACTCAAAAT TTAGTCACCA    900
CTTGCTTAGT TTCACCCCTA TGGGTAATCT TTTGACTTCA AACATGCGAT GTTTTTCTGG    960
CTTCGATGCA ATAGGCATAA AAGATCTTGA ACCTCTAAGC CGCGGCATGC ACAGTTGCTT   1020
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CCCAGTACAT CATGATGTTG TAACTAAGAT ATATCTTTAT TTGAGAACTC TCAAGAAGCC 1080
AGATAAGGAG TCTGCCGAGG CAAAGCTTCG ACAACTCATA GAAAAACCCA CAGGGAGGGA 1140
GATAAAGTTT ATCGAGGATT TTTCCTCACT AGTAATAAAT TGTGGGAGGA GTGGCTCTTT 1200
GCTTATGCCC AACATTTCTA AGTTGGTCAT ATCATTCTTT TGCCGGATGA TGCCAAATGC 1260
ACTCGCCAGG CTCTCTTCTA GCTTTCGAGA GTGTTCGCTA GATTCATTTG TGTACTCACT 1320
TGAGCCCTTT AATTTTTCCG TTAATTTAGT GGATATAACT CCTGATTTCT TTGAGCATTT 1380
ATTTCTCTTC TCCTGCCTAA ATGAGTTGAT CGAGGAGGAC GTTGAAGAGG TCATGGACAA 1440
TTCTTGGTTT GGACTTGGGG ACTTACAATT CAATCGCCAG AGGGCCCCGT TCTTTCTTGG 1500
GTCTTCATAT TGGCTCAACT CCAAATTTTC AGTTGAGCAC AAGTTTTCAG GCACCATCAA 1560
TTCTCAAATC ATGCAAGTTA TTTTATCTTT GATCCCATTT TCTGATGATC CCACTTTTAG 1620
GCCATCTTCT ACAGAGGTTA ACCTTGCACT ATCAGAGGTT AAGGCTGCGC TAGAAGCTAC 1680
TGGGCAGTCA AAATTGTTCA GGTTTTTGGT GGACGACTGT GCTATGCGTG AGGTTAGAAG 1740
TTCCTATAAG GTGGGCCTTT TTAAGCACAT AAAAGCCCTC ACTCATTGCT TTAATTCTTG 1800
TGGCCTCCAA TGGTTCCTCC TTAGGCAAAG GTCCAACCTC AAATTTCTGA AGGACAGGGC 1860
ATCGTCCTTT GCTGATCTTG ATTGTGAGGT TATCAAAGTT TATCAGCTTG TAACATCACA 1920
GGCAATACTT CCTGAGGCTC TGCTTAGCTT GACCAAAGTC TTTGTCAGGG ATTCTGACTC 1980
AAAGGGTGTT TCCATTCCCA GATTGGTCTC GAGAAATGAG CTAGAGGAAC TAGCTCACCC 2040
AGCTAATTCA GCCCTTGAGG AGCCTCAATC AGTTGATTGT AATGCAGGCA GGGTTCAAGC 2100
AAGCGTTTCA AGTTCCCAGC AGCTTGCCGA CACCCACTCT CTTGGTAGCG TTAAGTCATC 2160
AATTGAGACA GCTAACAAGG CTTTTAACTT GGAGGAGCTA AGGATCATGA TTAGAGTCTT 2220
GCCGGAGGAT TTTAACTGGG TGGCGAAGAA CATTGGTTTT AAAGACAGGC TGAGAGGCAG 2280
GGGTGCATCA TTCTTCTCAA AACCAGGAAT TTCATGTCAT AGTTACAATG GTGGGAGCCA 2340
CACAAGCTTA GGGTGGCCAA AGTTCATGGA TCAGATTCTA AGCTCCACTG GTGGACGTAA 2400
TTACTACAAT TCATGCCTGG CTCAGATCTA TGAGGAAAAT TCAAAATTGG CTCTTCATAA 2460
GGATGATGAG AGTTGCTATG AAATTGGGCA CAAAGTTTTG ACTGTTAATT TAATCGGCTC 2520
AGCAACTTTC ACTATTAGTA AGTCGCGAAA TTTGGTTGGG GGTAATCATT GCAGCCTGAC 2580
AATTGGGCCA AATGAGTTTT TCGAAATGCC TAGGGGCATG CAATGCAATT ACTTCCATGG 2640
GGTTTCCAAT TGTACGCCAG GGCGGGTATC GCTGACCTTT AGGCGCCAAA AGTTGGAAGA 2700
TGATGATTTG ATCTTCATAA ATCCACAGGT GCCCATTGAG CTCAATCATG AAAAGCTTGA 2760
CCGAAGTATG TGGCAGATGG GCCTTCATGG AATTAAGAAA TCTATTTCTA TGAATGGCAC 2820

GAGTTTTACC TCAGACCTAT GCTCTTGTTT CTCTTGCCAC AACTTTCATA AATTCAAGGA 2880

TCTCATCAAT AACTTGAGAT TGGCCCTAGG AGCACAAGGG CTAGGTCAGT GTGACAGGGT 2940

TGTGTTTGCA ACAACAGGTC CTGGTCTATC TAAGGTTTTA GAAATGCCTC GGAGCAAAAA 3000

GCAATCAATT TTGGTTCTTG AAGGTGCCCT ATCCATAGAA ACAGATTATG GTCCAAAAGT 3060

CCTGGGGTCT TTTGAAGTTT TCAAAGGGGA CTTTCACATT AAGAAGATGG AGGAAGGTTC 3120

AATTTTTGTA ATAACGTACA AGGCCCCAAT TAGATCCACT GGCAGGTTGA GGGTTCACAG 3180

TTCAGAATGC TCATTTTCCG GATCCAAAGA GGTATTGCTA GGCTGCCAGA TTGAGGCATG 3240

TGCTGATTAT GATATTGATG ATTTTAACAC TTTCTCTGTG CCTGGTGATG GCAATTGCTT 3300

TTGGCATTCT GTTGGTTTTT TACTTAGCAC TGATGGACTT GCCCTAAAGG CCGGTATTCG 3360

ATCTTTCGTG GAGAGTGAGC GCTTGGTAAG TCCAGATCTT TCAGCCCCAG CAATTTCTAA 3420

ACAATTGGAA GAGAATGCTT ATGCCGAGAA TGAGATGATC GCATTATTCT GCATTCGGCA 3480

CCACGTAAGG CCTATAGTGA TCACACCAGA ATATGAAGTT AGTTGGAAAT TCGGGGAAGG 3540

TGAGTGGCCC CTATGTGGAA TTCTTTGCCT TAAATCAAAT CACTTCCAAC CATGCGCCCC 3600

ACTGAATGGT TGCATGATCA CAGCCATTGC TTCAGCACTT GGAAGGCGTG AAGTTGATGT 3660

GTTAAATTAT CTGTGTAGAC CCAGCACTAA TCATATTTTT GAGGAGCTTT GTCAGGGAGG 3720

GGGCCTTAAC ATGATGTATT TAGCTGAAGC TTTTGAGGCC TTTGACATTT GCGCTAAATG 3780

TGATATAAAT GGAGAGATTG AAGTGATTAA TCCGTGTGGT AAAATTTCTG CATTGTTTGA 3840

CATAACTAAT GAGCACATAA GGCATGTTGA GAAAATAGGT AATGGCCCTC AGAGCATAAA 3900

AGTGGATGAA TTGCGGAAGG TCAAGCGATC CGCCCTCGAT TTCCTTTCAA TGAATGGGTC 3960

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TGTTTCTCCC AGACGTGCTT TAGCAAATTC AATCAAAAAT GATCTTGAAA TGGATGATAG 4260

CTGCAAAGTT GCTAAAGCAG GTAGGTCAAA GAAGGAAGGG TGGGATGTAG TAACTTTTGA 4320

GGTTTTCCTT AGAAAAGTTG CAGGATTGAA GGCTGGCCAC TGTGTGATTT TTGATGAGGT 4380

CCAGTTGTTT CCTCCTGGAT ACATCGATCT ATGCTTGCTT ATTATACGTA GTGATGCTTT 4440

CATTTCACTT GCTGGTGATC CATGTCAAAG CACATATGAC TCGCAAAAGG ATCGGGCAAT 4500

TTTGGGCGCT GAGCAGAGTG ACATACTTAG ACTGCTTGAG GGCAAAACGT ATAGGTATAA 4560

CATAGAAAGC AGGAGGTTTG TGAACCCAAT GTTCGAATCA AGACTGCCAT GTCACTTCAA 4620

AAAGGGCTCG ATGACTGCCG CTTTCGCTGA TTATGCAATC TTCCATAATA TGCATGACTT 4680
TCTCCTGGCG AGGTCAAAAG GTCCCTTGGA TGCCGTTTTG GTTTCCAGTT TTGAGGAGAA 4740
AAAGATAGTC CAGTCCTACT TTGGAATGAA ACAGCTCACA CTCACATTTG GTGAATCAAC 4800
TGGGTTGAAT TTCAAAAATG GGGGAATTCT CATATCACAT GATTCCTTTC ACACAGATGA 4860
TCGGCGGTGG CTTACTGCTT TATCTCGCTT CAGCCACAAT TTGGATTTGG TGAACATCAC 4920
AGGTCTGAGG GTGGAAAGTT TTCTCTCGCA CTTTGCTGGC AAACCCCTCT ACCATTTTTT 4980
AACAGCCAAA AGTGGGGAGA ATGTCATACG AGATTTGCTC CCAGGTGAGC CTAACTTCTT 5040
CAGTGGCTTT AACGTTAGCA TTGGAAAGAA TGAAGGTGTT AGGGAGGAGA AGTTATGTGG 5100
TGACCCATGG TTAAAAGTTA TGCTTTTCCT GGGTCAAGAT GAGGATTGTG AAGTTGAAGA 5160
GATGGAGTCA GAATGCTCAA ATGAAGAATG GTTTAAAACC CACATCCCCT TGAGTAATCT 5220
GGAGTCAACC AGGGCCAGGT GGGTGGGTAA AATGGCCTTG AAAGAGTATC GGGAGGTGCG 5280
TTGTGGTTAT GAAATGACTC AACAATTCTT TGATGAGCAT AGGGGTGGAA CTGGTGAGCA 5340
ACTGAGCAAT GCATGTGAGA GGTTTGAAAG CATTTACCCA AGGCATAAAG GAAATGATTC 5400
AATAACCTTC CTCATGGCTG TCCGAAAGCG TCTCAAATTT TCGAAGCCCC AGGTTGAAGC 5460
TGCCAAACTG AGGCGGGCCA AACGATATGG GAAATTCTTA TTAGATTCTT TCCTATCCAA 5520
AATCCCATTG AAAGCCAGTC ATAATTCCAT CATGTTTCAT GAAGCGGTAC AGGAGTTTGA 5580
GGCGAAGAAG GCTAGTAAGA GTGCAGCAAC TATAGAGAAT CATGCAGGTA GGTCATGCAG 5640
GGATTGGTTA TTAGATGTTG CTCTGATTTT TATGAAGTCA CAACACTGTA CTAAATTTGA 5700
CAACAGGCTT AGAGTAGCTA AAGCTGGGCA AACCCTTGCT TGCTTCCAAC ATGCTGTTCT 5760
GGTTCGCTTT GCACCCTATA TGAGATACAT TGAGAAAAAG CTAATGCAAG CTCTGAAGCC 5820
TAACTTCTAC ATCCATTCAG GGAAAGGTCT GACGAGCTGA ACGAGTGGGT CAGAACTAGA 5880
GGATTCACTG GAATTTGCAC AGAATCAGAC TACGAAGCCT TTGATGCTTC CCAAGACCAC 5940
TTCATCCTAG CATTCGAATT GCAGATAATG AAATTTTTGG GGTTACCTGA AGATTTAATT 6000
TTGGACTATG AATTCATAAA AATTCATTTG GGATCAAAGC TCGGATCATT CTCTATAATG 6060
AGGTTTACTG GGGAGGCCAG CACATTTCTG TTTAACACTA TGGCTAACAT GTTGTTCACC 6120
TTTCTGAGGT ACGAACTAAC AGGCTCTGAG TCAATAGCAT TTGCAGGTGA TGACATGTGT 6180
GCTAATCGAA GGTTGCGGCT TAAAACAGAG CATGAGGGTT TTCTGAACAT GATTTGCCTT 6240
AAGGCCAAGG TTCAGTTTGT TTCCAATCCC ACATTCTGCG GATGGTGTTT ATTTAAGGAA 6300
GGGATCTTCA AGAAGCCTCA ATTAATCTGG GAGCGGATAT GCATTGCTAG GGAGATGGGC 6360
AACCTGGAGA ATTGTATTGA CAATTATGCG ATAGAGGTCT CCTATGCATA CCGACTGGGA 6420

GAGCTAGCCA TTGAAATGAT GACCGAGGAA GAAGTGGAGG CCCATTATAA TTGTGTTAGA 6480
TTCTTGGTCA GGAACAAGCA TAAGATGAGA TGCTCAATTT CAGGCCTATT TGAAGCTATT 6540
GATTAGGCCT TAAGTATTTG GCATTATTTG AGTATTATGA ATAATTTAGT TAAAGCATTG 6600
TCAGCATTTG AGTTTGTAGG TGTTTTCAGT GTGCTTAAAT TTCCAGTAGT CATTCATAGT 6660
GTGCCTGGTA GTGGTAAAAG TAGTTTAATA AGGGAGCTAA TTTCCGAGGA TGAGAATTTC 6720
ATAGCTTTCA CAGCAGGTGT TCCAGACAGC CCTAATCTCA CAGGAAGGTA CATTAAGCCT 6780
TATTCTCCAG GGTGTGCAGT GCCAGGGAAA GTTAATATAC TTGATGAGTA CTTGTCCGTC 6840
CAAGATTTTT CAGGTTTTGA TGTGCTGTTC TCGGACCCAT ACCAAAACAT CAGCATTCCT 6900
AAAGAGGCAC ATTTCATCAA GTCAAAAACT TGTAGGTTTG GCGTGAATAC TTGCAAATAT 6960
CTTTCCTCCT TCGGTTTTAA GGTTAGCAGT GACGGTTTGG ACAAAGTCAT TGTGGGGTCG 7020
CCTTTTACAC TAGATGTTGA AGGGGTGCTA ATATGCTTTG GTAAGGAGGC AGTGGATCTC 7080
GCTGTTGCGC ACAACTCTGA ATTCAAATTA CCTTGTGAAG TTAGAGGTTC AACTTTTAAC 7140
GTCGTAACTC TTTTGAAATC AAGAGATCCA ACCCCAGAGG ATAGGCACTG GTTTTACATT 7200
GCTGCTACAA GACACAGGGA GAAATTGATA ATCATGCAGT AAGATGCCTT TTCAGCAGCC 7260
TGCGAATTGG GCAAAAACCA TAACTCCATT GACAGTTGGC TTGGGCATTG GGCTTGTGCT 7320
GCATTTTCTG AGGAAGTCAA ATCTACCTTA TTCAGGGGAC AACATCCATC AATTCCCTCA 7380
CGGTGGGCGT TACAGGGACG GTACAAAAAG TATAACTTAC TGTGGTCCAA AGCAATCCTT 7440
CCCCAGCTCT GGGATATTCG GCCAATCTGA GAATTTTGTG CCCTTAATGC TTGTCATAGG 7500
TCTAATCGCA TTCATACATG TATTGTCTGT TTGGAATTCT GGTCTTGGTA GGAATTGTAA 7560
TTGCCATCCA AATCCTTGCT CATGTAGACA GCAGTAGTGG CAACCACCAA GGTTGCTTCA 7620
TTAGGGCCAC TGGAGAGTCA ATTTTGATTG AAAACTGCGG CCCAAGTGAG GCCCTTGCAT 7680
CCACTGTGAA GGAGGTGCTG GGAGGTTTGA AGGCTTTAGG GGTTAGCCGT GCTGTTGAAG 7740
AAATTGATTA TCATTGTTAA ATTGGCTGAA TGGCAAGTCA AATTGGGAAA CTCCCCGGTG 7800
AATCAAATGA GGCTTTTGAA GCCCGGCTAA AATCGCTGGA GTTAGCTAGA GCTCAAAAGC 7860
AGCCGGAAGG TTCTAATGCA CCACCTACTC TCAGTGGCAT TCTTGCCAAA CGCAAGAGGA 7920
TTATAGAGAA TGCACTTTCA AAGACGGTGG ACATGAGGGA GGTTTTGAAA CACGAAACGG 7980
TGGTGATTTC CCCAAATGTC ATGGATGAAG GTGCAATAGA CGAGCTGATT CGTGCATTTG 8040
GTGAATCTGG CATAGCTGAA AGCGTGCAAT TTGATGTGGC CATAGATATA GCACGTCACT 8100
GCTCTGATGT TGGTAGCTCC CAGAGGTCAA CCCTGATTGG CAAGAGTCCA TTTTGTGACC 8160
TAAACAGATC AGAAATAGCT GGGATTATAA GGGAGGTGAC CACATTACGT AGATTTTGCA 8220

TGTACTATGC AAAAATCGTG TGGAACATCC ATCTGGAGAC GGGGATACCA CCAGCTAACT 8280

GGGCCAAGAA AGGATTTAAT GAGAATGAAA AGTTTGCAGC CTTTGATTTT TTCTTGGGAG 8340

TCACAGATGA GAGTGCGCTT GAACCAAAGG GTGGAATTAA AAGAGCTCCA ACGAAAGCTG 8400

AGATGGTTGC TAATATCGCC TCTTTTGAGG TTCAAGTGCT CAGACAAGCT ATGGCTGAAG 8460

GCAAGCGGAG TTCCAACCTT GGAGAGATTA GTGGTGGAAC GGCTGGTGCA CTCATCAACA 8520

ACCCCTTTTC AAATGTTACA CATGAATGAG GATGACGAAG TCAGCGACAA TTCCGCAGTC 8580

CAATAATTCC CCGATTTCAA GGCTGGGTTA AGCCTGTTCG CTGGAATACC GTACTAATAG 8640

TATTCCCTTT CCATGCTAAA TCCTATTTAA TATATAAGGT GTGGAAAGTA AAAGAAGATT 8700

TGGTGTGTTT TTATAGTTTT CATTCAAAAA AAAAAAAAAA AAA 8743

The DNA molecule of SEQ. ID. No. 1 contains at least five open reading frames (e.g., ORF1-ORF5), each of which encodes a particular protein or polypeptide of RSPaV-1, and a 3' untranscribed region downstream of ORF5.

Another DNA molecule of the present invention (RSPaV-1 ORF1) includes nucleotides 62-6547 of SEQ. ID. No. 1. The DNA molecule of RSPaV-1 ORF1 encodes for a RSPaV-1 replicase and comprises a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

ATGGCCCTCT CTTATAGGCC TGCTGTTGAA GAGGTGCTCG CAAAATTCAC CTCTGATGAA 60

CAATCCAGGG TTTCTGCTAC AGCTCTCAAG GCATTAGTAG ACTTAGAGGA AAGTCAGCAC 120

AATTTGTTCT CTTTCGCATT GCCTGATAGA AGCAAAGAAA GGCTGATATC TTCTGGCATT 180

TACTTAAGTC CTTACAGTTT CAGACCCCAC TCACATCCAG TTTGTAAAAC TTTAGAAAAT 240

CACATTTTGT ACAATGTTTT ACCTAGTTAT GTTAATAATT CATTTTACTT TGTAGGAATC 300

AAGGATTTTA AGCTGCAGTT CTTGAAAAGG AGGAATAAGG ATCTCAGCTT GGTAGCACTC 360

ATAAATAGGT TTGTGACAAG TCGTGATGTT AGTAGGTATG GGTCTGAGTT CGTTATAAGT 420

TCTAGTGACA AATCAAGTCA GGTTGTCAGT AGAAAGGGCA TTGGTGATTC TAACACACTC 480

CGGAGATTGG TCCCACGTGT AATTTCCACA GGTGCCAGGA ATCTTTTTCT GCATGATGAG 540

ATTCACTACT GGTCAATTAG TGATCTGATC AATTTTTTGG ACGTTGCCAA GCCAAGCATG 600

CTCTTGGCAA CTGCAGTAAT CCCTCCAGAA GTGCTGGTTG GCTCTCCAGA GAGTCTTAAC 660

CCTTGGGCCT ACCAGTATAA AATCAATGGC AACCAACTGC TCTTCGCACC AGATGGCAAC 720

TGGAATGAGA TGTACTCACA ACCTTTGTCA TGCAGATACC TGCTCAAGGC CAGATCTGTA 780

GTTCTGCCCG ATGGCTCACG CTACTCGGTT GACATCATTC ACTCAAAATT TAGTCACCAC 840

TTGCTTAGTT TCACCCCTAT GGGTAATCTT TTGACTTCAA ACATGCGATG TTTTTCTGGC 900

TTCGATGCAA TAGGCATAAA AGATCTTGAA CCTCTAAGCC GCGGCATGCA CAGTTGCTTC 960

CCAGTACATC ATGATGTTGT AACTAAGATA TATCTTTATT TGAGAACTCT CAAGAAGCCA 1020

GATAAGGAGT CTGCCGAGGC AAAGCTTCGA CAACTCATAG AAAAACCCAC AGGGAGGGAG 1080

ATAAAGTTTA TCGAGGATTT TTCCTCACTA GTAATAAATT GTGGGAGGAG TGGCTCTTTG 1140

CTTATGCCCA ACATTTCTAA GTTGGTCATA TCATTCTTTT GCCGGATGAT GCCAAATGCA 1200

CTCGCCAGGC TCTCTTCTAG CTTTCGAGAG TGTTCGCTAG ATTCATTTGT GTACTCACTT 1260

GAGCCCTTTA ATTTTTCCGT TAATTTAGTG GATATAACTC CTGATTTCTT TGAGCATTTA 1320

TTTCTCTTCT CCTGCCTAAA TGAGTTGATC GAGGAGGACG TTGAAGAGGT CATGGACAAT 1380

TCTTGGTTTG GACTTGGGGA CTTACAATTC AATCGCCAGA GGGCCCCGTT CTTTCTTGGG 1440

TCTTCATATT GGCTCAACTC CAAATTTTCA GTTGAGCACA AGTTTTCAGG CACCATCAAT 1500

TCTCAAATCA TGCAAGTTAT TTTATCTTTG ATCCCATTTT CTGATGATCC CACTTTTAGG 1560

CCATCTTCTA CAGAGGTTAA CCTTGCACTA TCAGAGGTTA AGGCTGCGCT AGAAGCTACT 1620

GGGCAGTCAA AATTGTTCAG GTTTTTGGTG GACGACTGTG CTATGCGTGA GGTTAGAAGT 1680

TCCTATAAGG TGGGCCTTTT TAAGCACATA AAAGCCCTCA CTCATTGCTT TAATTCTTGT 1740

GGCCTCCAAT GGTTCCTCCT TAGGCAAAGG TCCAACCTCA AATTTCTGAA GGACAGGGCA 1800

TCGTCCTTTG CTGATCTTGA TTGTGAGGTT ATCAAAGTTT ATCAGCTTGT AACATCACAG 1860

GCAATACTTC CTGAGGCTCT GCTTAGCTTG ACCAAAGTCT TTGTCAGGGA TTCTGACTCA 1920

AAGGGTGTTT CCATTCCCAG ATTGGTCTCG AGAAATGAGC TAGAGGAACT AGCTCACCCA 1980

GCTAATTCAG CCCTTGAGGA GCCTCAATCA GTTGATTGTA ATGCAGGCAG GGTTCAAGCA 2040

AGCGTTTCAA GTTCCCAGCA GCTTGCCGAC ACCCACTCTC TTGGTAGCGT TAAGTCATCA 2100

ATTGAGACAG CTAACAAGGC TTTTAACTTG GAGGAGCTAA GGATCATGAT TAGAGTCTTG 2160

CCGGAGGATT TTAACTGGGT GGCGAAGAAC ATTGGTTTTA AAGACAGGCT GAGAGGCAGG 2220

GGTGCATCAT TCTTCTCAAA ACCAGGAATT TCATGTCATA GTTACAATGG TGGGAGCCAC 2280

ACAAGCTTAG GGTGGCCAAA GTTCATGGAT CAGATTCTAA GCTCCACTGG TGGACGTAAT 2340

TACTACAATT CATGCCTGGC TCAGATCTAT GAGGAAAATT CAAAATTGGC TCTTCATAAG 2400

GATGATGAGA GTTGCTATGA AATTGGGCAC AAAGTTTTGA CTGTTAATTT AATCGGCTCA 2460

GCAACTTTCA CTATTAGTAA GTCGCGAAAT TTGGTTGGGG GTAATCATTG CAGCCTGACA 2520

ATTGGGCCAA ATGAGTTTTT CGAAATGCCT AGGGGCATGC AATGCAATTA CTTCCATGGG 2580

GTTTCCAATT GTACGCCAGG GCGGGTATCG CTGACCTTTA GGCGCCAAAA GTTGGAAGAT 2640
GATGATTTGA TCTTCATAAA TCCACAGGTG CCCATTGAGC TCAATCATGA AAAGCTTGAC 2700
CGAAGTATGT GGCAGATGGG CCTTCATGGA ATTAAGAAAT CTATTTCTAT GAATGGCACG 2760
AGTTTTACCT CAGACCTATG CTCTTGTTTC TCTTGCCACA ACTTTCATAA ATTCAAGGAT 2820
CTCATCAATA ACTTGAGATT GGCCCTAGGA GCACAAGGGC TAGGTCAGTG TGACAGGGTT 2880
GTGTTTGCAA CAACAGGTCC TGGTCTATCT AAGGTTTTAG AAATGCCTCG GAGCAAAAAG 2940
CAATCAATTT TGGTTCTTGA AGGTGCCCTA TCCATAGAAA CAGATTATGG TCCAAAAGTC 3000
CTGGGGTCTT TTGAAGTTTT CAAAGGGGAC TTTCACATTA AGAAGATGGA GGAAGGTTCA 3060
ATTTTTGTAA TAACGTACAA GGCCCCAATT AGATCCACTG GCAGGTTGAG GGTTCACAGT 3120
TCAGAATGCT CATTTTCCGG ATCCAAAGAG GTATTGCTAG GCTGCCAGAT TGAGGCATGT 3180
GCTGATTATG ATATTGATGA TTTTAACACT TTCTCTGTGC CTGGTGATGG CAATTGCTTT 3240
TGGCATTCTG TTGGTTTTTT ACTTAGCACT GATGGACTTG CCCTAAAGGC CGGTATTCGA 3300
TCTTTCGTGG AGAGTGAGCG CTTGGTAAGT CCAGATCTTT CAGCCCCAGC AATTTCTAAA 3360
CAATTGGAAG AGAATGCTTA TGCCGAGAAT GAGATGATCG CATTATTCTG CATTCGGCAC 3420
CACGTAAGGC CTATAGTGAT CACACCAGAA TATGAAGTTA GTTGGAAATT CGGGGAAGGT 3480
GAGTGGCCCC TATGTGGAAT TCTTTGCCTT AAATCAAATC ACTTCCAACC ATGCGCCCCA 3540
CTGAATGGTT GCATGATCAC AGCCATTGCT TCAGCACTTG GAAGGCGTGA AGTTGATGTG 3600
TTAAATTATC TGTGTAGACC CAGCACTAAT CATATTTTTG AGGAGCTTTG TCAGGGAGGG 3660
GGCCTTAACA TGATGTATTT AGCTGAAGCT TTTGAGGCCT TTGACATTTG CGCTAAATGT 3720
GATATAAATG GAGAGATTGA AGTGATTAAT CCGTGTGGTA AAATTTCTGC ATTGTTTGAC 3780
ATAACTAATG AGCACATAAG GCATGTTGAG AAAATAGGTA ATGGCCCTCA GAGCATAAAA 3840
GTGGATGAAT TGCGGAAGGT CAAGCGATCC GCCCTCGATT TCCTTTCAAT GAATGGGTCT 3900
AAAATAACCT ACTTCCCAAG CTTTGAGCGG GCTGAAAAGT TGCAAGGATG TTTGCTAGGG 3960
GGCCTAACTG GCGTTATAAG TGATGAGAAG TTCAGTGATG CAAAACCTTG GCTTTCTGGT 4020
ATATCTACTA CTGATATTAA GCCAAGGGAA TTGACTGTCG TGCTTGGTAC ATTTGGGGCT 4080
GGGAAGAGTT TCTTGTACAA GAGTTTCATG AAAAGGTCTG AGGGTAAATT CGTAACCTTT 4140
GTTTCTCCCA GACGTGCTTT AGCAAATTCA ATCAAAAATG ATCTTGAAAT GGATGATAGC 4200
TGCAAAGTTG CTAAAGCAGG TAGGTCAAAG AAGGAAGGGT GGGATGTAGT AACTTTTGAG 4260
GTTTTCCTTA GAAAAGTTGC AGGATTGAAG GCTGGCCACT GTGTGATTTT TGATGAGGTC 4320
CAGTTGTTTC CTCCTGGATA CATCGATCTA TGCTTGCTTA TTATACGTAG TGATGCTTTC 4380

ATTTCACTTG CTGGTGATCC ATGTCAAAGC ACATATGACT CGCAAAAGGA TCGGGCAATT 4440
TTGGGCGCTG AGCAGAGTGA CATACTTAGA CTGCTTGAGG GCAAAACGTA TAGGTATAAC 4500
ATAGAAAGCA GGAGGTTTGT GAACCCAATG TTCGAATCAA GACTGCCATG TCACTTCAAA 4560
AAGGGCTCGA TGACTGCCGC TTTCGCTGAT TATGCAATCT TCCATAATAT GCATGACTTT 4620
CTCCTGGCGA GGTCAAAAGG TCCCTTGGAT GCCGTTTTGG TTTCCAGTTT TGAGGAGAAA 4680
AAGATAGTCC AGTCCTACTT TGGAATGAAA CAGCTCACAC TCACATTTGG TGAATCAACT 4740
GGGTTGAATT TCAAAAATGG GGGAATTCTC ATATCACATG ATTCCTTTCA CACAGATGAT 4800
CGGCGGTGGC TTACTGCTTT ATCTCGCTTC AGCCACAATT TGGATTTGGT GAACATCACA 4860
GGTCTGAGGG TGGAAAGTTT TCTCTCGCAC TTTGCTGGCA AACCCCTCTA CCATTTTTTA 4920
ACAGCCAAAA GTGGGGAGAA TGTCATACGA GATTTGCTCC CAGGTGAGCC TAACTTCTTC 4980
AGTGGCTTTA ACGTTAGCAT TGGAAAGAAT GAAGGTGTTA GGGAGGAGAA GTTATGTGGT 5040
GACCCATGGT TAAAAGTTAT GCTTTTCCTG GGTCAAGATG AGGATTGTGA AGTTGAAGAG 5100
ATGGAGTCAG AATGCTCAAA TGAAGAATGG TTTAAAACCC ACATCCCCTT GAGTAATCTG 5160
GAGTCAACCA GGGCCAGGTG GGTGGGTAAA ATGGCCTTGA AAGAGTATCG GGAGGTGCGT 5220
TGTGGTTATG AAATGACTCA ACAATTCTTT GATGAGCATA GGGGTGGAAC TGGTGAGCAA 5280
CTGAGCAATG CATGTGAGAG GTTTGAAAGC ATTTACCCAA GGCATAAAGG AAATGATTCA 5340
ATAACCTTCC TCATGGCTGT CCGAAAGCGT CTCAAATTTT CGAAGCCCCA GGTTGAAGCT 5400
GCCAAACTGA GGCGGGCCAA ACCATATGGG AAATTCTTAT TAGATTCTTT CCTATCCAAA 5460
ATCCCATTGA AAGCCAGTCA TAATTCCATC ATGTTTCATG AAGCGGTACA GGAGTTTGAG 5520
GCGAAGAAGG CTAGTAAGAG TGCAGCAACT ATAGAGAATC ATGCAGGTAG GTCATGCAGG 5580
GATTGGTTAT TAGATGTTGC TCTGATTTTT ATGAAGTCAC AACACTGTAC TAAATTTGAC 5640
AACAGGCTTA GAGTAGCTAA AGCTGGGCAA ACCCTTGCTT GCTTCCAACA TGCTGTTCTG 5700
GTTCGCTTTG CACCCTATAT GAGATACATT GAGAAAAAGC TAATGCAAGC TCTGAAGCCT 5760
AACTTCTACA TCCATTCAGG GAAAGGTCTG ACGAGCTGAA CGAGTGGGTC AGAACTAGAG 5820
GATTCACTGG AATTTGCACA GAATCAGACT ACGAAGCCTT TGATGCTTCC CAAGACCACT 5880
TCATCCTAGC ATTCGAATTG CAGATAATGA AATTTTTGGG GTTACCTGAA GATTTAATTT 5940
TGGACTATGA ATTCATAAAA ATTCATTTGG GATCAAAGCT CGGATCATTC TCTATAATGA 6000
GGTTTACTGG GGAGGCCAGC ACATTTCTGT TTAACACTAT GGCTAACATG TTGTTCACCT 6060
TTCTGAGGTA CGAACTAACA GGCTCTGAGT CAATAGCATT TGCAGGTGAT GACATGTGTG 6120
CTAATCGAAG GTTGCGGCTT AAAACAGAGC ATGAGGGTTT TCTGAACATG ATTTGCCTTA 6180

AGGCCAAGGT TCAGTTTGTT TCCAATCCCA CATTCTGCGG ATGGTGTTTA TTTAAGGAAG 6240

GGATCTTCAA GAAGCCTCAA TTAATCTGGG AGCGGATATG CATTGCTAGG GAGATGGGCA 6300

ACCTGGAGAA TTGTATTGAC AATTATGCGA TAGAGGTCTC CTATGCATAC CGACTGGGAG 6360

AGCTAGCCAT TGAAATGATG ACCGAGGAAG AAGTGGAGGC CCATTATAAT TGTGTTAGAT 6420

TCTTGGTCAG GAACAAGCAT AAGATGAGAT GCTCAATTTC AGGCCTATTT GAAGCTATTG 6480

ATTAG 6485

The RSPaV-1 replicase has a deduced amino acid sequence corresponding to SEQ. ID. No. 3 as follows:

Met Ala Leu Ser Tyr Arg Pro Ala Val Glu Glu Val Leu Ala Lys Phe
1 5 10 15

Thr Ser Asp Glu Gln Ser Arg Val Ser Ala Thr Ala Leu Lys Ala Leu
20 25 30

Val Asp Leu Glu Glu Ser Gln His Asn Leu Phe Ser Phe Ala Leu Pro
35 40 45

Asp Arg Ser Lys Glu Arg Leu Ile Ser Ser Gly Ile Tyr Leu Ser Pro
50 55 60

Tyr Ser Phe Arg Pro His Ser His Pro Val Cys Lys Thr Leu Glu Asn
65 70 75 80

His Ile Leu Tyr Asn Val Leu Pro Ser Tyr Val Asn Asn Ser Phe Tyr
85 90 95

Phe Val Gly Ile Lys Asp Phe Lys Leu Gln Phe Leu Lys Arg Arg Asn
100 105 110

Lys Asp Leu Ser Leu Val Ala Leu Ile Asn Arg Phe Val Thr Ser Arg
115 120 125

Asp Val Ser Arg Tyr Gly Ser Glu Phe Val Ile Ser Ser Ser Asp Lys
130 135 140

Ser Ser Gln Val Val Ser Arg Lys Gly Ile Gly Asp Ser Asn Thr Leu
145 150 155 160

Arg Arg Leu Val Pro Arg Val Ile Ser Thr Gly Ala Arg Asn Leu Phe
165 170 175

Leu His Asp Glu Ile His Tyr Trp Ser Ile Ser Asp Leu Ile Asn Phe
180 185 190

Leu Asp Val Ala Lys Pro Ser Met Leu Leu Ala Thr Ala Val Ile Pro
195 200 205

Pro Glu Val Leu Val Gly Ser Pro Glu Ser Leu Asn Pro Trp Ala Tyr
210 215 220

Gln Tyr Lys Ile Asn Gly Asn Gln Leu Leu Phe Ala Pro Asp Gly Asn
225 230 235 240

Trp Asn Glu Met Tyr Ser Gln Pro Leu Ser Cys Arg Tyr Leu Leu Lys
245 250 255

Ala Arg Ser Val Val Leu Pro Asp Gly Ser Arg Tyr Ser Val Asp Ile
260 265 270

Ile His Ser Lys Phe Ser His His Leu Leu Ser Phe Thr Pro Met Gly
275 280 285

Asn Leu Leu Thr Ser Asn Met Arg Cys Phe Ser Gly Phe Asp Ala Ile
290 295 300

Gly Ile Lys Asp Leu Glu Pro Leu Ser Arg Gly Met His Ser Cys Phe
305 310 315 320

Pro Val His His Asp Val Val Thr Lys Ile Tyr Leu Tyr Leu Arg Thr
325 330 335

Leu Lys Lys Pro Asp Lys Glu Ser Ala Glu Ala Lys Leu Arg Gln Leu
340 345 350

Ile Glu Lys Pro Thr Gly Arg Glu Ile Lys Phe Ile Glu Asp Phe Ser
355 360 365

Ser Leu Val Ile Asn Cys Gly Arg Ser Gly Ser Leu Leu Met Pro Asn
370 375 380

Ile Ser Lys Leu Val Ile Ser Phe Phe Cys Arg Met Met Pro Asn Ala
385 390 395 400

Leu Ala Arg Leu Ser Ser Ser Phe Arg Glu Cys Ser Leu Asp Ser Phe
405 410 415

Val Tyr Ser Leu Glu Pro Phe Asn Phe Ser Val Asn Leu Val Asp Ile
420 425 430

Thr Pro Asp Phe Phe Glu His Leu Phe Leu Phe Ser Cys Leu Asn Glu
435 440 445

Leu Ile Glu Glu Asp Val Glu Glu Val Met Asp Asn Ser Trp Phe Gly
450 455 460

Leu Gly Asp Leu Gln Phe Asn Arg Gln Arg Ala Pro Phe Phe Leu Gly
465 470 475 480

Ser Ser Tyr Trp Leu Asn Ser Lys Phe Ser Val Glu His Lys Phe Ser
485 490 495

Gly Thr Ile Asn Ser Gln Ile Met Gln Val Ile Leu Ser Leu Ile Pro
500 505 510

Phe Ser Asp Asp Pro Thr Phe Arg Pro Ser Ser Thr Glu Val Asn Leu
515 520 525

Ala Leu Ser Glu Val Lys Ala Ala Leu Glu Ala Thr Gly Gln Ser Lys
530 535 540

Leu Phe Arg Phe Leu Val Asp Asp Cys Ala Met Arg Glu Val Arg Ser
545 550 555 560

Ser Tyr Lys Val Gly Leu Phe Lys His Ile Lys Ala Leu Thr His Cys
565 570 575

Phe Asn Ser Cys Gly Leu Gln Trp Phe Leu Leu Arg Gln Arg Ser Asn
580 585 590

Leu Lys Phe Leu Lys Asp Arg Ala Ser Ser Phe Ala Asp Leu Asp Cys
595 600 605

Glu Val Ile Lys Val Tyr Gln Leu Val Thr Ser Gln Ala Ile Leu Pro
610 615 620

Glu Ala Leu Leu Ser Leu Thr Lys Val Phe Val Arg Asp Ser Asp Ser
625 630 635 640

Lys Gly Val Ser Ile Pro Arg Leu Val Ser Arg Asn Glu Leu Glu Glu
645 650 655

Leu Ala His Pro Ala Asn Ser Ala Leu Glu Glu Pro Gln Ser Val Asp
660 665 670

Cys Asn Ala Gly Arg Val Gln Ala Ser Val Ser Ser Ser Gln Gln Leu
675 680 685

Ala Asp Thr His Ser Leu Gly Ser Val Lys Ser Ser Ile Glu Thr Ala
690 695 700

Asn Lys Ala Phe Asn Leu Glu Glu Leu Arg Ile Met Ile Arg Val Leu
705 710 715 720

Pro Glu Asp Phe Asn Trp Val Ala Lys Asn Ile Gly Phe Lys Asp Arg
725 730 735

Leu Arg Gly Arg Gly Ala Ser Phe Phe Ser Lys Pro Gly Ile Ser Cys
740 745 750

His Ser Tyr Asn Gly Gly Ser His Thr Ser Leu Gly Trp Pro Lys Phe
755 760 765

Met Asp Gln Ile Leu Ser Ser Thr Gly Gly Arg Asn Tyr Tyr Asn Ser
770 775 780

Cys Leu Ala Gln Ile Tyr Glu Glu Asn Ser Lys Leu Ala Leu His Lys
785 790 795 800

Asp Asp Glu Ser Cys Tyr Glu Ile Gly His Lys Val Leu Thr Val Asn
805 810 815

Leu Ile Gly Ser Ala Thr Phe Thr Ile Ser Lys Ser Arg Asn Leu Val
820 825 830

Gly Gly Asn His Cys Ser Leu Thr Ile Gly Pro Asn Glu Phe Phe Glu
835 840 845

Met Pro Arg Gly Met Gln Cys Asn Tyr Phe His Gly Val Ser Asn Cys
850 855 860

Thr Pro Gly Arg Val Ser Leu Thr Phe Arg Arg Gln Lys Leu Glu Asp
865 870 875 880

Asp Asp Leu Ile Phe Ile Asn Pro Gln Val Pro Ile Glu Leu Asn His
885 890 895

Glu Lys Leu Asp Arg Ser Met Trp Gln Met Gly Leu His Gly Ile Lys
900 905 910

Lys Ser Ile Ser Met Asn Gly Thr Ser Phe Thr Ser Asp Leu Cys Ser
915 920 925

Cys Phe Ser Cys His Asn Phe His Lys Phe Lys Asp Leu Ile Asn Asn
930 935 940

Leu Arg Leu Ala Leu Gly Ala Gln Gly Leu Gly Gln Cys Asp Arg Val
945 950 955 960

Val Phe Ala Thr Thr Gly Pro Gly Leu Ser Lys Val Leu Glu Met Pro
965 970 975

Arg Ser Lys Lys Gln Ser Ile Leu Val Leu Glu Gly Ala Leu Ser Ile
980 985 990

Glu Thr Asp Tyr Gly Pro Lys Val Leu Gly Ser Phe Glu Val Phe Lys
995 1000 1005

Gly Asp Phe His Ile Lys Lys Met Glu Glu Gly Ser Ile Phe Val Ile
1010 1015 1020

Thr Tyr Lys Ala Pro Ile Arg Ser Thr Gly Arg Leu Arg Val His Ser
1025 1030 1035 1040

Ser Glu Cys Ser Phe Ser Gly Ser Lys Glu Val Leu Leu Gly Cys Gln
1045 1050 1055

Ile Glu Ala Cys Ala Asp Tyr Asp Ile Asp Asp Phe Asn Thr Phe Ser
1060 1065 1070

Val Pro Gly Asp Gly Asn Cys Phe Trp His Ser Val Gly Phe Leu Leu
1075 1080 1085

Ser Thr Asp Gly Leu Ala Leu Lys Ala Gly Ile Arg Ser Phe Val Glu
1090 1095 1100

Ser Glu Arg Leu Val Ser Pro Asp Leu Ser Ala Pro Ala Ile Ser Lys
1105 1110 1115 1120

Gln Leu Glu Glu Asn Ala Tyr Ala Glu Asn Glu Met Ile Ala Leu Phe
1125 1130 1135

Cys Ile Arg His His Val Arg Pro Ile Val Ile Thr Pro Glu Tyr Glu
1140 1145 1150

Val Ser Trp Lys Phe Gly Glu Gly Glu Trp Pro Leu Cys Gly Ile Leu
1155 1160 1165

Cys Leu Lys Ser Asn His Phe Gln Pro Cys Ala Pro Leu Asn Gly Cys
1170 1175 1180

Met Ile Thr Ala Ile Ala Ser Ala Leu Gly Arg Arg Glu Val Asp Val
1185 1190 1195 1200

Leu Asn Tyr Leu Cys Arg Pro Ser Thr Asn His Ile Phe Glu Glu Leu
1205 1210 1215

Cys Gln Gly Gly Gly Leu Asn Met Met Tyr Leu Ala Glu Ala Phe Glu
1220 1225 1230

Ala Phe Asp Ile Cys Ala Lys Cys Asp Ile Asn Gly Glu Ile Glu Val
1235 1240 1245

Ile Asn Pro Cys Gly Lys Ile Ser Ala Leu Phe Asp Ile Thr Asn Glu
1250 1255 1260

His Ile Arg His Val Glu Lys Ile Gly Asn Gly Pro Gln Ser Ile Lys
1265 1270 1275 1280

Val Asp Glu Leu Arg Lys Val Lys Arg Ser Ala Leu Asp Phe Leu Ser
1285 1290 1295

Met Asn Gly Ser Lys Ile Thr Tyr Phe Pro Ser Phe Glu Arg Ala Glu
1300 1305 1310

Lys Leu Gln Gly Cys Leu Leu Gly Gly Leu Thr Gly Val Ile Ser Asp
1315 1320 1325

Glu Lys Phe Ser Asp Ala Lys Pro Trp Leu Ser Gly Ile Ser Thr Thr
1330 1335 1340

Asp Ile Lys Pro Arg Glu Leu Thr Val Val Leu Gly Thr Phe Gly Ala
1345 1350 1355 1360

Gly Lys Ser Phe Leu Tyr Lys Ser Phe Met Lys Arg Ser Glu Gly Lys
1365 1370 1375

Phe Val Thr Phe Val Ser Pro Arg Arg Ala Leu Ala Asn Ser Ile Lys
1380 1385 1390

Asn Asp Leu Glu Met Asp Asp Ser Cys Lys Val Ala Lys Ala Gly Arg
1395 1400 1405

Ser Lys Lys Glu Gly Trp Asp Val Val Thr Phe Glu Val Phe Leu Arg
1410 1415 1420

Lys Val Ala Gly Leu Lys Ala Gly His Cys Val Ile Phe Asp Glu Val
1425 1430 1435 1440

Gln Leu Phe Pro Pro Gly Tyr Ile Asp Leu Cys Leu Leu Ile Ile Arg
1445 1450 1455

Ser Asp Ala Phe Ile Ser Leu Ala Gly Asp Pro Cys Gln Ser Thr Tyr
1460 1465 1470

Asp Ser Gln Lys Asp Arg Ala Ile Leu Gly Ala Glu Gln Ser Asp Ile
1475 1480 1485

Leu Arg Leu Leu Glu Gly Lys Thr Tyr Arg Tyr Asn Ile Glu Ser Arg
1490 1495 1500

Arg Phe Val Asn Pro Met Phe Glu Ser Arg Leu Pro Cys His Phe Lys
1505 1510 1515 1520

Lys Gly Ser Met Thr Ala Ala Phe Ala Asp Tyr Ala Ile Phe His Asn
1525 1530 1535

Met His Asp Phe Leu Leu Ala Arg Ser Lys Gly Pro Leu Asp Ala Val
1540 1545 1550

Leu Val Ser Ser Phe Glu Glu Lys Lys Ile Val Gln Ser Tyr Phe Gly
1555 1560 1565

Met Lys Gln Leu Thr Leu Thr Phe Gly Glu Ser Thr Gly Leu Asn Phe
1570 1575 1580

Lys Asn Gly Gly Ile Leu Ile Ser His Asp Ser Phe His Thr Asp Asp
1585 1590 1595 1600

Arg Arg Trp Leu Thr Ala Leu Ser Arg Phe Ser His Asn Leu Asp Leu
1605 1610 1615

Val Asn Ile Thr Gly Leu Arg Val Glu Ser Phe Leu Ser His Phe Ala
1620 1625 1630

Gly Lys Pro Leu Tyr His Phe Leu Thr Ala Lys Ser Gly Glu Asn Val
1635 1640 1645

Ile Arg Asp Leu Leu Pro Gly Glu Pro Asn Phe Phe Ser Gly Phe Asn
1650 1655 1660

Val Ser Ile Gly Lys Asn Glu Gly Val Arg Glu Glu Lys Leu Cys Gly
1665 1670 1675 1680

Asp Pro Trp Leu Lys Val Met Leu Phe Leu Gly Gln Asp Glu Asp Cys
1685 1690 1695

Glu Val Glu Glu Met Glu Ser Glu Cys Ser Asn Glu Glu Trp Phe Lys
1700 1705 1710

Thr His Ile Pro Leu Ser Asn Leu Glu Ser Thr Arg Ala Arg Trp Val
1715 1720 1725

Gly Lys Met Ala Leu Lys Glu Tyr Arg Glu Val Arg Cys Gly Tyr Glu
1730 1735 1740

Met Thr Gln Gln Phe Phe Asp Glu His Arg Gly Gly Thr Gly Glu Gln
1745 1750 1755 1760

Leu Ser Asn Ala Cys Glu Arg Phe Glu Ser Ile Tyr Pro Arg His Lys
1765 1770 1775

Gly Asn Asp Ser Ile Thr Phe Leu Met Ala Val Arg Lys Arg Leu Lys
1780 1785 1790

Phe Ser Lys Pro Gln Val Glu Ala Ala Lys Leu Arg Arg Ala Lys Pro
1795 1800 1805

Tyr Gly Lys Phe Leu Leu Asp Ser Phe Leu Ser Lys Ile Pro Leu Lys
1810 1815 1820

Ala Ser His Asn Ser Ile Met Phe His Glu Ala Val Gln Glu Phe Glu
1825 1830 1835 1840

Ala Lys Lys Ala Ser Lys Ser Ala Ala Thr Ile Glu Asn His Ala Gly
1845 1850 1855

Arg Ser Cys Arg Asp Trp Leu Leu Asp Val Ala Leu Ile Phe Met Lys
1860 1865 1870

Ser Gln His Cys Thr Lys Phe Asp Asn Arg Leu Arg Val Ala Lys Ala
1875 1880 1885

Gly Gln Thr Leu Ala Cys Phe Gln His Ala Val Leu Val Arg Phe Ala
1890 1895 1900

Pro Tyr Met Arg Tyr Ile Glu Lys Lys Leu Met Gln Ala Leu Lys Pro
1905 1910 1915 1920

Asn Phe Tyr Ile His Ser Gly Lys Gly Leu Asp Glu Leu Asn Glu Trp
1925 1930 1935

Val Arg Thr Arg Gly Phe Thr Gly Ile Cys Thr Glu Ser Asp Tyr Glu
1940 1945 1950

Ala Phe Asp Ala Ser Gln Asp His Phe Ile Leu Ala Phe Glu Leu Gln
1955 1960 1965

Ile Met Lys Phe Leu Gly Leu Pro Glu Asp Leu Ile Leu Asp Tyr Glu
1970 1975 1980

Phe Ile Lys Ile His Leu Gly Ser Lys Leu Gly Ser Phe Ser Ile Met
1985 1990 1995 2000

Arg Phe Thr Gly Glu Ala Ser Thr Phe Leu Phe Asn Thr Met Ala Asn
2005 2010 2015

Met Leu Phe Thr Phe Leu Arg Tyr Glu Leu Thr Gly Ser Glu Ser Ile
2020 2025 2030

Ala Phe Ala Gly Asp Asp Met Cys Ala Asn Arg Arg Leu Arg Leu Lys
2035 2040 2045

Thr Glu His Glu Gly Phe Leu Asn Met Ile Cys Leu Lys Ala Lys Val
2050 2055 2060

Gln Phe Val Ser Asn Pro Thr Phe Cys Gly Trp Cys Leu Phe Lys Glu
2065 2070 2075 2080

Gly Ile Phe Lys Lys Pro Gln Leu Ile Trp Glu Arg Ile Cys Ile Ala
2085 2090 2095

Arg Glu Met Gly Asn Leu Glu Asn Cys Ile Asp Asn Tyr Ala Ile Glu
2100 2105 2110

Val Ser Tyr Ala Tyr Arg Leu Gly Glu Leu Ala Ile Glu Met Met Thr
2115 2120 2125

Glu Glu Glu Val Glu Ala His Tyr Asn Cys Val Arg Phe Leu Val Arg
2130 2135 2140

```
Asn Lys His Lys Met Arg Cys Ser Ile Ser Gly Leu Phe Glu Ala Ile
2145                2150                2155                2160

Asp
```

The replicase of SEQ. ID. No. 3 has a molecular weight of about 240 to 246 kDa, preferably about 244 kDa.

Another DNA molecule of the present invention (RSPaV-1 ORF2) includes nucleotides 6578-7243 of SEQ. ID. No. 1. The DNA molecule of RSPaV-1 ORF2 encodes for a first protein or polypeptide of an RSPaV-1 triple gene block and comprises a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

```
ATGAATAATT TAGTTAAAGC ATTGTCAGCA TTTGAGTTTG TAGGTGTTTT CAGTGTGCTT     60
AAATTTCCAG TAGTCATTCA TAGTGTGCCT GGTAGTGGTA AAAGTAGTTT AATAAGGGAG    120
CTAATTTCCG AGGATGAGAA TTTCATAGCT TTCACAGCAG GTGTTCCAGA CAGCCCTAAT    180
CTCACAGGAA GGTACATTAA GCCTTATTCT CCAGGGTGTG CAGTGCCAGG GAAAGTTAAT    240
ATACTTGATG AGTACTTGTC CGTCCAAGAT TTTTCAGGTT TTGATGTGCT GTTCTCGGAC    300
CCATACCAAA ACATCAGCAT TCCTAAAGAG GCACATTTCA TCAAGTCAAA AACTTGTAGG    360
TTTGGCGTGA ATACTTGCAA ATATCTTTCC TCCTTCGGTT TTAAGGTTAG CAGTGACGGT    420
TTGGACAAAG TCATTGTGGG GTCGCCTTTT ACACTAGATG TTGAAGGGGT GCTAATATGC    480
TTTGGTAAGG AGGCAGTGGA TCTCGCTGTT GCGCACAACT CTGAATTCAA ATTACCTTGT    540
GAAGTTAGAG GTTCAACTTT TAACGTCGTA ACTCTTTTGA AATCAAGAGA TCCAACCCCA    600
GAGGATAGGC ACTGGTTTTA CATTGCTGCT ACAAGACACA GGGAGAAATT GATAATCATG    660
CAG                                                                  663
```

The first protein or polypeptide of the RSPaV-1 triple gene block has a deduced amino acid sequence corresponding to SEQ. ID. No. 5 as follows:

```
Met Asn Asn Leu Val Lys Ala Leu Ser Ala Phe Glu Phe Val Gly Val
1               5                   10                  15

Phe Ser Val Leu Lys Phe Pro Val Val Ile His Ser Val Pro Gly Ser
            20                  25                  30

Gly Lys Ser Ser Leu Ile Arg Glu Leu Ile Ser Glu Asp Glu Asn Phe
        35                  40                  45

Ile Ala Phe Thr Ala Gly Val Pro Asp Ser Pro Asn Leu Thr Gly Arg
    50                  55                  60
```

```
Tyr Ile Lys Pro Tyr Ser Pro Gly Cys Ala Val Pro Gly Lys Val Asn
65                  70                  75                  80

Ile Leu Asp Glu Tyr Leu Ser Val Gln Asp Phe Ser Gly Phe Asp Val
                85                  90                  95

Leu Phe Ser Asp Pro Tyr Gln Asn Ile Ser Ile Pro Lys Glu Ala His
            100                 105                 110

Phe Ile Lys Ser Lys Thr Cys Arg Phe Gly Val Asn Thr Cys Lys Tyr
        115                 120                 125

Leu Ser Ser Phe Gly Phe Lys Val Ser Ser Asp Gly Leu Asp Lys Val
    130                 135                 140

Ile Val Gly Ser Pro Phe Thr Leu Asp Val Glu Gly Val Leu Ile Cys
145                 150                 155                 160

Phe Gly Lys Glu Ala Val Asp Leu Ala Val Ala His Asn Ser Glu Phe
                165                 170                 175

Lys Leu Pro Cys Glu Val Arg Gly Ser Thr Phe Asn Val Val Thr Leu
            180                 185                 190

Leu Lys Ser Arg Asp Pro Thr Pro Glu Asp Arg His Trp Phe Tyr Ile
        195                 200                 205

Ala Ala Thr Arg His Arg Glu Lys Leu Ile Ile Met Gln
    210                 215                 220
```

The first protein or polypeptide of the RSPaV-1 triple gene block has a molecular weight of about 20 to 26 kDa, preferably 24.4 kDa.

Another DNA molecule of the present invention (RSPaV-1 ORF3) includes nucleotides 7245-7598 of SEQ. ID. No. 1. The DNA molecule of RSPaV-1 ORF3 encodes for a second protein or polypeptide of the triple gene block and comprises a nucleotide sequence corresponding to SEQ. ID. No. 6 as follows:

```
ATGCCTTTTC AGCAGCCTGC GAATTGGGCA AAAACCATAA CTCCATTGAC AGTTGGCTTG      60
GGCATTGGGC TTGTGCTGCA TTTTCTGAGG AAGTCAAATC TACCTTATTC AGGGGACAAC     120
ATCCATCAAT TCCCTCACGG TGGGCGTTAC AGGGACGGTA CAAAAAGTAT AACTTACTGT     180
GGTCCAAAGC AATCCTTCCC CAGCTCTGGG ATATTCGGCC AATCTGAGAA TTTTGTGCCC     240
TTAATGCTTG TCATAGGTCT AATCGCATTC ATACATGTAT TGTCTGTTTG GAATTCTGGT     300
CTTGGTAGGA ATTGTAATTG CCATCCAAAT CCTTGCTCAT GTAGACAGCA G              351
```

The second protein or polypeptide of the RSPaV-1 triple gene block has a deduced amino acid sequence corresponding to SEQ. ID. No. 7 as follows:

Met Pro Phe Gln Gln Pro Ala Asn Trp Ala Lys Thr Ile Thr Pro Leu
1 5 10 15

Thr Val Gly Leu Gly Ile Gly Leu Val Leu His Phe Leu Arg Lys Ser
20 25 30

Asn Leu Pro Tyr Ser Gly Asp Asn Ile His Gln Phe Pro His Gly Gly
35 40 45

Arg Tyr Arg Asp Gly Thr Lys Ser Ile Thr Tyr Cys Gly Pro Lys Gln
50 55 60

Ser Phe Pro Ser Ser Gly Ile Phe Gly Gln Ser Glu Asn Phe Val Pro
65 70 75 80

Leu Met Leu Val Ile Gly Leu Ile Ala Phe Ile His Val Leu Ser Val
85 90 95

Trp Asn Ser Gly Leu Gly Arg Asn Cys Asn Cys His Pro Asn Pro Cys
100 105 110

Ser Cys Arg Gln Gln
115

The second protein or polypeptide of the RSPaV-1 triple gene block has a molecular weight of about 10 to 15 kDa, preferably 12.8 kDa.

Yet another DNA molecule of the present invention (RSPaV-1 ORF4) includes nucleotides 7519-7761 of SEQ. ID. No. 1. The DNA molecule of RSPaV-1 ORF4 encodes for a third protein or polypeptide of the RSPaV-1 triple gene block and comprises a nucleotide sequence corresponding to SEQ. ID. No. 8 as follows:

ATGTATTGTC TGTTTGGAAT TCTGGTCTTG GTAGGAATTG TAATTGCCAT CCAAATCCTT 60

GCTCATGTAG ACAGCAGTAG TGGCAACCAC CAAGGTTGCT TCATTAGGGC CACTGGAGAG 120

TCAATTTTGA TTGAAAACTG CGGCCCAAGT GAGGCCCTTG CATCCACTGT GAAGGAGGTG 180

CTGGGAGGTT TGAAGGCTTT AGGGGTTAGC CGTGCTGTTG AAGAAATTGA TTATCATTGT 240

The third protein or polypeptide of the RSPaV-1 triple gene block has a deduced amino acid sequence corresponding to SEQ. ID. No. 9 as follows:

Met Tyr Cys Leu Phe Gly Ile Leu Val Leu Val Gly Ile Val Ile Ala
1 5 10 15

Ile Gln Ile Leu Ala His Val Asp Ser Ser Ser Gly Asn His Gln Gly
20 25 30

Cys Phe Ile Arg Ala Thr Gly Glu Ser Ile Leu Ile Glu Asn Cys Gly
35 40 45

```
Pro Ser Glu Ala Leu Ala Ser Thr Val Lys Glu Val Leu Gly Gly Leu
    50                  55                  60

Lys Ala Leu Gly Val Ser Arg Ala Val Glu Glu Ile Asp Tyr His Cys
65                  70                  75                  80
```

The third protein or polypeptide of the RSPaV-1 triple gene block has a molecular weight of about 5 to 10 kDa, preferably 8.4 kDa.

Still another DNA molecule of the present invention (RSPaV-1 ORF5) includes nucleotides 7771-8550 of SEQ. ID. No. 1. The DNA molecule of RSPaV-1 ORF5 encodes for a RSPaV-1 coat protein and comprises a nucleotide sequence corresponding to SEQ. ID. No. 10 as follows:

```
ATGGCAAGTC AAATTGGGAA ACTCCCCGGT GAATCAAATG AGGCTTTTGA AGCCCGGCTA     60
AAATCGCTGG AGTTAGCTAG AGCTCAAAAG CAGCCGGAAG GTTCTAATGC ACCACCTACT    120
CTCAGTGGCA TTCTTGCCAA ACGCAAGAGG ATTATAGAGA ATGCACTTTC AAAGACGGTG    180
GACATGAGGG AGGTTTTGAA ACACGAAACG GTGGTGATTT CCCCAAATGT CATGGATGAA    240
GGTGCAATAG ACGAGCTGAT TCGTGCATTT GGTGAATCTG GCATAGCTGA AAGCGTGCAA    300
TTTGATGTGG CCATAGATAT AGCACGTCAC TGCTCTGATG TTGGTAGCTC CCAGAGTTCA    360
ACCCTGATTG GCAAGAGTCC ATTTTGTGAC CTAAACAGAT CAGAAATAGC TGGGATTATA    420
AGGGAGGTGA CCACATTACG TAGATTTTGC ATGTACTATG CAAAAATCGT GTGGAACATC    480
CATCTGGAGA CGGGGATACC ACCAGCTAAC TGGGCCAAGA AAGGATTTAA TGAGAATGAA    540
AAGTTTGCAG CCTTTGATTT TTTCTTGGGA GTCACAGATG AGAGTGCGCT TGAACCAAAG    600
GGTGGAATTA AAAGAGCTCC AACGAAAGCT GAGATGGTTG CTAATATCGC CTCTTTTGAG    660
GTTCAAGTGC TCAGACAAGC TATGGCTGAA GGCAAGCGGA GTTCCAACCT TGGAGAGATT    720
AGTGGTGGAA CGGCTGGTGC ACTCATCAAC AACCCCTTTT CAAATGTTAC ACATGAA       777
```

The RSPaV-1 coat protein has a deduced amino acid sequence corresponding to SEQ. ID. No. 11 as follows:

```
Met Ala Ser Gln Ile Gly Lys Leu Pro Gly Glu Ser Asn Glu Ala Phe
1               5                   10                  15

Glu Ala Arg Leu Lys Ser Leu Glu Leu Ala Arg Ala Gln Lys Gln Pro
            20                  25                  30

Glu Gly Ser Asn Ala Pro Pro Thr Leu Ser Gly Ile Leu Ala Lys Arg
        35                  40                  45
```

```
Lys Arg Ile Ile Glu Asn Ala Leu Ser Lys Thr Val Asp Met Arg Glu
    50                  55                  60

Val Leu Lys His Glu Thr Val Val Ile Ser Pro Asn Val Met Asp Glu
65                  70                  75                  80

Gly Ala Ile Asp Glu Leu Ile Arg Ala Phe Gly Glu Ser Gly Ile Ala
                85                  90                  95

Glu Ser Val Gln Phe Asp Val Ala Ile Asp Ile Ala Arg His Cys Ser
            100                 105                 110

Asp Val Gly Ser Ser Gln Ser Ser Thr Leu Ile Gly Lys Ser Pro Phe
        115                 120                 125

Cys Asp Leu Asn Arg Ser Glu Ile Ala Gly Ile Ile Arg Glu Val Thr
    130                 135                 140

Thr Leu Arg Arg Phe Cys Met Tyr Tyr Ala Lys Ile Val Trp Asn Ile
145                 150                 155                 160

His Leu Glu Thr Gly Ile Pro Pro Ala Asn Trp Ala Lys Lys Gly Phe
                165                 170                 175

Asn Glu Asn Glu Lys Phe Ala Ala Phe Asp Phe Phe Leu Gly Val Thr
            180                 185                 190

Asp Glu Ser Ala Leu Glu Pro Lys Gly Gly Ile Lys Arg Ala Pro Thr
        195                 200                 205

Lys Ala Glu Met Val Ala Asn Ile Ala Ser Phe Glu Val Gln Val Leu
    210                 215                 220

Arg Gln Ala Met Ala Glu Gly Lys Arg Ser Ser Asn Leu Gly Glu Ile
225                 230                 235                 240

Ser Gly Gly Thr Ala Gly Ala Leu Ile Asn Asn Pro Phe Ser Asn Val
                245                 250                 255

Thr His Glu
```

The RSPaV-1 coat protein has a molecular weight of about 25 to 30 kDa, preferably 28 kDa.

The DNA molecule which constitutes the substantial portion of the RSPaV strain RSP47-4 genome comprises the nucleotide sequence corresponding to SEQ. ID. No. 12 as follows:

```
GGCTGGGCAA ACTTTGGCCT GCTTTCAACA CGCCGTCTTG GTTCGCTTTG CACCCTACAT     60

GCGATACATT GAAAAGAAGC TTGTGCAGGC ATTGAAACCA AATTTCTACA TTCATTCTGG    120

CAAAGGTCTT GATGAGCTAA GTGAATGGGT TAGAGCCAGA GGTTTCACAG GTGTGTGTAC    180

TGAGTCAGAC TATGAAGCTT TTGATGCATC CCAAGATCAT TTCATCCTGG CATTTGAACT    240
```

GCAAATCATG AGATTTTTAG GACTGCCAGA AGATCTGATT TTAGATTATG AGTTCATCAA 300

AATTCATCTT GGGTCAAAGC TTGGCTCTTT TGCAATTATG AGATTCACAG GTGAGGCAAG 360

CACCTTCCTA TTCAATACTA TGGCCAACAT GCTATTCACT TTCCTGAGGT ATGAGTTGAC 420

AGGTTCTGAA TCAATTGCAT TTGCTGGAGA TGATATGTGT GCTAATCGCA GGTTAAGACT 480

CAAGACTGAG CACGCCGGCT TTCTAAACAT GATCTGTCTC AAAGCTAAGG TGCAGTTTGT 540

CACAAATCCC ACCTTCTGTG GATGGTGTTT GTTTAAAGAG GGAATCTTTA AAAAACCCCA 600

GCTCATTTGG GAAAGGATCT GCATTGCTAG GGAAATGGGT AACTTGGACA ATTGCATTGA 660

CAATTACGCA ATTGAGGTGT CTTATGCTTA CAGACTTGGG GAATTGTCCA TAGGCGTGAT 720

GACTGAGGAG GAAGTTGAAG CACATTCTAA CTGCGTGCGT TTCCTGGTTC GCAATAAGCA 780

CAAGATGAGG TGCTCAATTT CTGGTTTGTT TGAAGTAATT GTTTAGGCCT TAAGTGTTTG 840

GCATGGTGTG AGTATTATGA ATAACTTAGT CAAAGCTTTG TCTGCTTTTG AATTTGTTGG 900

TGTGTTTTGT GTACTTAAAT TTCCAGTTGT TGTTCACAGT GTTCCAGGTA GCGGTAAAAG 960

TAGCCTAATA AGGGAGCTCA TTTCTGAAGA CGAGGCTTTT GTGGCCTTTA CAGCAGGTGT 1020

GCCAGACAGT CCAAATCTGA CAGGGAGGTA CATCAAGCCC TACGCTCCAG GGTGTGCAGT 1080

GCAAGGGAAA ATAAACATAC TTGATGAGTA CTTGTCTGTC TCTGATACTT CTGGCTTTGA 1140

TGTGCTGTTC TCAGACCCTT ACCAGAATGT CAGCATTCCA AGGGAGGCAC ACTTCATAAA 1200

AACCAAAACC TGTAGGTTTG GTACCAACAC CTGCAAGTAC CTTCAATCTT TTGGCTTTAA 1260

TGTTTGTAGT GATGGGGTGG ATAAAGTTGT TGTAGGGTCG CCATTTGAAC TGGAGGTTGA 1320

GGGGGTTCTC ATTTGCTTTG GAAAGGAGGC TGTAGATCTA GCAGTTGCAC ACAATTCTGA 1380

CTTCAAGTTG CCCTGCGAGG TGCGGGGTTC AACATTTGAC GTTGTAACGT TATTGAAGTC 1440

CAGGGATCCA ACTTCAGAAG ATAAGCATTG GTTCTACGTT GCAGCCACAA GGCATCGAAG 1500

TAAACTGATA ATAATGCAGT AAAATGCCTT TTCAGCAACC TGCCAACTGG GCTAAGACCA 1560

TAACTCCATT AACTATTGGT TTGGGCATTG GGTTGGTTCT GCACTTCTTA AGGAAATCAA 1620

ATCTGCCATA TTCAGGAGAC AATATTCACC AGTTCCCACA CGGAGGGCAT TACAGGGACG 1680

GCACGAAGAG TATAACCTAT TGTGGCCCTA GGCAGTCATT CCCAAGCTCA GGAATATTCG 1740

GTCAGTCTGA AAATTTCGTA CCTCTAATAT TGGTCGTGAC TCTGGTCGCT TTTATACATG 1800

CGTTATCTCT TTGGAATTCT GGTCCTAGTA GGAGTTGCAA TTGCCATCCA AATCCTTGCA 1860

CATGTAGACA GCAGTAGTGG CAACCATCAA GGCTGTTTCA TAAGAGCCAC CGGGGAGTCA 1920

ATAGTAATTG AGAATTGTGG GCCGAGCGAG GCCCTAGCTG CTACAGTCAA AGAGGTGTTG 1980

GGCGGTCTAA AGGCTTTAGG GGTTAGCCAA AAGGTTGATG AAATTAATTA CAGTTGTTGA 2040

GACAGTTGAA TGGCAAGTCA AGTTGGAAAA TTGCCTGGCG AATCAAATGA AGCATATGAG 2100

GCTAGACTCA AGGCTTTAGA GTTAGCAAGG GCCCAAAAAG CTCCAGAAGT CTCCAACCAA 2160

CCTCCCACAC TTGGAGGCAT TCTAGCCAAA AGGAAAAGAG TGATTGAGAA TGCACTCTCA 2220

AAGACAGTGG ATATGCGTGA AGTCTTAAGG CATGAATCTG TTGTACTCTC CCCGAATGTA 2280

ATGGACGAGG GAGCAATAGA CGAGCTGATT CGTGCCTTTG GGGAGTCGGG CATAGCTGAA 2340

AATGTGCAGT TTGATGTTGC AATAGACATT GCTCGCCACT GTTCTGATGT GGGGAGCTCT 2400

CAGAGGTCAA CCCTTATTGG TAAAAGCCCC TTCTGTGAGT TAAATAGGTC TGAAATTGCC 2460

GGAATAATAA GGGAGGTGAC CACGCTGCGC AGATTTTGCA TGTACTACGC AAAGATTGTG 2520

TGGAACATCC ATTTGGAGAC GGGAATACCA CCAGCTAATT GGGCCAAGAA AGGATTTAAT 2580

GAGAATGAAA AGTTTGCAGC CTTTGACTTC TTCCTTGGAG TCACAGATGA AAGCGCGCTT 2640

GAGCCTAAGG GTGGAGTCAA GAGAGCTCCA ACAAAAGCAG 2680

The RSP47-4 strain contains five open reading frames (i.e., ORF1-5). ORF1 and ORF5 are only partially sequenced. RSP47-4 is 79% identical in nucleotide sequence to the corresponding region of RSPaV-1. The amino acid sequence identities between the corresponding ORFs of RSP47-4 and RSPaV-1 are: 94.1% for ORF1, 88.2% for ORF2, 88.9% for ORF3, 86.2% for ORF4, and 92.9% for ORF5. The nucleotide sequences of the five potential ORFs of RSP47-4 are given below.

Another DNA molecule of the present invention (RSP47-4 incomplete ORF1) includes nucleotides 1-768 of SEQ. ID. No. 12. This DNA molecule is believed to code for a polypeptide portion of a RSP47-4 replicase and comprises a nucleotide sequence corresponding to SEQ. ID. No. 13 as follows:

ATGCGATACA TTGAAAAGAA GCTTGTGCAG GCATTGAAAC CAAATTTCTA CATTCATTCT 60

GGCAAAGGTC TTGATGAGCT AAGTGAATGG GTTAGAGCCA GAGGTTTCAC AGGTGTGTGT 120

ACTGAGTCAG ACTATGAAGC TTTTGATGCA TCCCAAGATC ATTTCATCCT GGCATTTGAA 180

CTGCAAATCA TGAGATTTTT AGGACTGCCA GAAGATCTGA TTTTAGATTA TGAGTTCATC 240

AAAATTCATC TTGGGTCAAA GCTTGGCTCT TTTGCAATTA TGAGATTCAC AGGTGAGGCA 300

AGCACCTTCC TATTCAATAC TATGGCCAAC ATGCTATTCA CTTTCCTGAG GTATGAGTTG 360

ACAGGTTCTG AATCAATTGC ATTTGCTGGA GATGATATGT GTGCTAATCG CAGGTTAAGA 420

CTCAAGACTG AGCACGCCGG CTTTCTAAAC ATGATCTGTC TCAAAGCTAA GGTGCAGTTT 480

GTCACAAATC CCACCTTCTG TGGATGGTGT TTGTTTAAAG AGGGAATCTT TAAAAAACCC 540

CAGCTCATTT GGGAAAGGAT CTGCATTGCT AGGGAAATGG GTAACTTGGA CAATTGCATT 600

GACAATTACG CAATTGAGGT GTCTTATGCT TACAGACTTG GGGAATTGTC CATAGGCGTG 660

ATGACTGAGG AGGAAGTTGA AGCACATTCT AACTGCGTGC GTTTCCTGGT TCGCAATAAG 720

CACAAGATGA GGTGCTCAAT TTCTGGTTTG TTTGAAGTAA TTGTTTA 767

The polypeptide has a deduced amino acid sequence corresponding to SEQ. ID. No. 14 as follows:

```
Met Arg Tyr Ile Glu Lys Lys Leu Val Gln Ala Leu Lys Pro Asn Phe
1               5                   10                  15

Tyr Ile His Ser Gly Lys Gly Leu Asp Glu Leu Ser Glu Trp Val Arg
            20                  25                  30

Ala Arg Gly Phe Thr Gly Val Cys Thr Glu Ser Asp Tyr Glu Ala Phe
        35                  40                  45

Asp Ala Ser Gln Asp His Phe Ile Leu Ala Phe Glu Leu Gln Ile Met
    50                  55                  60

Arg Phe Leu Gly Leu Pro Glu Asp Leu Ile Leu Asp Tyr Glu Phe Ile
65                  70                  75                  80

Lys Ile His Leu Gly Ser Lys Leu Gly Ser Phe Ala Ile Met Arg Phe
                85                  90                  95

Thr Gly Glu Ala Ser Thr Phe Leu Phe Asn Thr Met Ala Asn Met Leu
            100                 105                 110

Phe Thr Phe Leu Arg Tyr Glu Leu Thr Gly Ser Glu Ser Ile Ala Phe
        115                 120                 125

Ala Gly Asp Asp Met Cys Ala Asn Arg Arg Leu Arg Leu Lys Thr Glu
    130                 135                 140

His Ala Gly Phe Leu Asn Met Ile Cys Leu Lys Ala Lys Val Gln Phe
145                 150                 155                 160

Val Thr Asn Pro Thr Phe Cys Gly Trp Cys Leu Phe Lys Glu Gly Ile
                165                 170                 175

Phe Lys Lys Pro Gln Leu Ile Trp Glu Arg Ile Cys Ile Ala Arg Glu
            180                 185                 190

Met Gly Asn Leu Asp Asn Cys Ile Asp Asn Tyr Ala Ile Glu Val Ser
        195                 200                 205

Tyr Ala Tyr Arg Leu Gly Glu Leu Ser Ile Gly Val Met Thr Glu Glu
    210                 215                 220

Glu Val Glu Ala His Ser Asn Cys Val Arg Phe Leu Val Arg Asn Lys
225                 230                 235                 240

His Lys Met Arg Cys Ser Ile Ser Gly Leu Phe Glu Val Ile Val
                245                 250                 255
```

Another DNA molecule of the present invention (RSP47-4 ORF2) includes nucleotides 857-1522 of SEQ. ID. No. 12. This DNA molecule codes for a first protein or polypeptide of an RSP47-4 triple gene block and comprises a nucleotide sequence corresponding to SEQ. ID. No. 15 as follows:

```
ATGAATAACT TAGTCAAAGC TTTGTCTGCT TTTGAATTTG TTGGTGTGTT TTGTGTACTT    60
AAATTTCCAG TTGTTGTTCA CAGTGTTCCA GGTAGCGGTA AAAGTAGCCT AATAAGGGAG   120
CTCATTTCTG AAGACGAGGC TTTTGTGGCC TTTACAGCAG GTGTGCCAGA CAGTCCAAAT   180
CTGACAGGGA GGTACATCAA GCCCTACGCT CCAGGGTGTG CAGTGCAAGG GAAAATAAAC   240
ATACTTGATG AGTACTTGTC TGTCTCTGAT ACTTCTGGCT TTGATGTGCT GTTCTCAGAC   300
CCTTACCAGA ATGTCAGCAT TCCAAGGGAG GCACACTTCA TAAAAACCAA AACCTGTAGG   360
TTTGGTACCA ACACCTGCAA GTACCTTCAA TCTTTTGGCT TTAATGTTTG TAGTGATGGG   420
GTGGATAAAG TTGTTGTAGG GTCGCCATTT GAACTGGAGG TTGAGGGGGT TCTCATTTGC   480
TTTGGAAAGG AGGCTGTAGA TCTAGCAGTT GCACACAATT CTGACTTCAA GTTGCCCTGC   540
GAGGTGCGGG GTTCAACATT TGACGTTGTA ACGTTATTGA AGTCCAGGGA TCCAACTTCA   600
GAAGATAAGC ATTGGTTCTA CGTTGCAGCC ACAAGGCATC GAAGTAAACT GATAATAATG   660
CAGTAA                                                              666
```

The first protein or polypeptide of the RSP47-4 triple gene block has a deduced amino acid sequence corresponding to SEQ. ID. No. 16 as follows:

```
Met Asn Asn Leu Val Lys Ala Leu Ser Ala Phe Glu Phe Val Gly Val
1               5                   10                  15

Phe Cys Val Leu Lys Phe Pro Val Val Val His Ser Val Pro Gly Ser
            20                  25                  30

Gly Lys Ser Ser Leu Ile Arg Glu Leu Ile Ser Glu Asp Glu Ala Phe
        35                  40                  45

Val Ala Phe Thr Ala Gly Val Pro Asp Ser Pro Asn Leu Thr Gly Arg
    50                  55                  60

Tyr Ile Lys Pro Tyr Ala Pro Gly Cys Ala Val Gln Gly Lys Ile Asn
65                  70                  75                  80

Ile Leu Asp Glu Tyr Leu Ser Val Ser Asp Thr Ser Gly Phe Asp Val
                85                  90                  95

Leu Phe Ser Asp Pro Tyr Gln Asn Val Ser Ile Pro Arg Glu Ala His
            100                 105                 110
```

```
Phe Ile Lys Thr Lys Thr Cys Arg Phe Gly Thr Asn Thr Cys Lys Tyr
        115                 120                 125

Leu Gln Ser Phe Gly Phe Asn Val Cys Ser Asp Gly Val Asp Lys Val
    130                 135                 140

Val Val Gly Ser Pro Phe Glu Leu Glu Val Glu Gly Val Leu Ile Cys
145                 150                 155                 160

Phe Gly Lys Glu Ala Val Asp Leu Ala Val Ala His Asn Ser Asp Phe
                165                 170                 175

Lys Leu Pro Cys Glu Val Arg Gly Ser Thr Phe Asp Val Val Thr Leu
            180                 185                 190

Leu Lys Ser Arg Asp Pro Thr Ser Glu Asp Lys His Trp Phe Tyr Val
        195                 200                 205

Ala Ala Thr Arg His Arg Ser Lys Leu Ile Ile Met Gln
    210                 215                 220
```

The first protein or polypeptide of the RSP47-4 triple gene block has a molecular weight of about 20 to 26 kDa., preferably 24.3 kDa.

Another DNA molecule of the present invention (RSP47-4 ORF3) includes nucleotides 1524-1877 of SEQ. ID. No. 12. This DNA molecule codes for a second protein or polypeptide of the RSP47-4 triple gene block and comprises a nucleotide sequence corresponding to SEQ. ID. No. 17 as follows:

```
ATGCCTTTTC AGCAACCTGC CAACTGGGCT AAGACCATAA CTCCATTAAC TATTGGTTTG     60

GGCATTGGGT TGGTTCTGCA CTTCTTAAGG AAATCAAATC TGCCATATTC AGGAGACAAT    120

ATTCACCAGT TCCCACACGG AGGGCATTAC AGGGACGGCA CGAAGAGTAT AACCTATTGT    180

GGCCCTAGGC AGTCATTCCC AAGCTCAGGA ATATTCGGTC AGTCTGAAAA TTTCGTACCT    240

CTAATATTGG TCGTGACTCT GGTCGCTTTT ATACATGCGT TATCTCTTTG GAATTCTGGT    300

CCTAGTAGGA GTTGCAATTG CCATCCAAAT CCTTGCACAT GTAGACAGCA GTAG          354
```

The second protein or polypeptide of the RSP47-4 triple gene block has a deduced amino acid sequence corresponding to SEQ. ID. No. 18 as follows:

```
Met Pro Phe Gln Gln Pro Ala Asn Trp Ala Lys Thr Ile Thr Pro Leu
1               5                   10                  15

Thr Ile Gly Leu Gly Ile Gly Leu Val Leu His Phe Leu Arg Lys Ser
            20                  25                  30

Asn Leu Pro Tyr Ser Gly Asp Asn Ile His Gln Phe Pro His Gly Gly
        35                  40                  45
```

His Tyr Arg Asp Gly Thr Lys Ser Ile Thr Tyr Cys Gly Pro Arg Gln
50 55 60

Ser Phe Pro Ser Ser Gly Ile Phe Gly Gln Ser Glu Asn Phe Val Pro
65 70 75 80

Leu Ile Leu Val Val Thr Leu Val Ala Phe Ile His Ala Leu Ser Leu
85 90 95

Trp Asn Ser Gly Pro Ser Arg Ser Cys Asn Cys His Pro Asn Pro Cys
100 105 110

Thr Cys Arg Gln Gln
115

The second protein or polypeptide of the RSP47-4 triple gene block has a molecular weight of about 10 to 15 kDa., preferably 12.9 kDa.

Another DNA molecule of the present invention (RSP47-4 ORF4) includes nucleotides 1798-2040 of SEQ. ID. No. 12. This DNA molecule codes for a third protein or polypeptide of the RSP47-4 triple gene block and comprises a nucleotide sequence corresponding to SEQ. ID. No. 19 as follows:

ATGCGTTATC TCTTTGGAAT TCTGGTCCTA GTAGGAGTTG CAATTGCCAT CCAAATCCTT 60

GCACATGTAG ACAGCAGTAG TGGCAACCAT CAAGGCTGTT TCATAAGAGC CACCGGGGAG 120

TCAATAGTAA TTGAGAATTG TGGGCCGAGC GAGGCCCTAG CTGCTACAGT CAAAGAGGTG 180

TTGGGCGGTC TAAAGGCTTT AGGGGTTAGC CAAAAGGTTG ATGAAATTAA TTACAGTTGT 240

TGA 243

The third protein or polypeptide of the RSP47-4 triple gene block has a deduced amino acid sequence corresponding to SEQ. ID. No. 20 as follows:

Met Arg Tyr Leu Phe Gly Ile Leu Val Leu Val Gly Val Ala Ile Ala
1 5 10 15

Ile Gln Ile Leu Ala His Val Asp Ser Ser Ser Gly Asn His Gln Gly
20 25 30

Cys Phe Ile Arg Ala Thr Gly Glu Ser Ile Val Ile Glu Asn Cys Gly
35 40 45

Pro Ser Glu Ala Leu Ala Ala Thr Val Lys Glu Val Leu Gly Gly Leu
50 55 60

Lys Ala Leu Gly Val Ser Gln Lys Val Asp Glu Ile Asn Tyr Ser Cys
65 70 75 80

The third protein or polypeptide of the RSP47-4 triple gene block has a molecular weight of about 5 to 10 kDa., preferably 8.3 kDa.

Yet another DNA molecule of the present invention (RSP47-4 ORF5) includes nucleotides 2050-2680 of SEQ. ID. No. 12. This DNA molecule codes for a partial RSP47-4 coat protein or polypeptide and comprises a nucleotide sequence corresponding to SEQ. ID. No. 21 as follows:

```
ATGGCAAGTC AAGTTGGAAA ATTGCCTGGC GAATCAAATG AAGCATATGA GGCTAGACTC    60
AAGGCTTTAG AGTTAGCAAG GGCCCAAAAA GCTCCAGAAG TCTCCAACCA ACCTCCCACA   120
CTTGGAGGCA TTCTAGCCAA AAGGAAAAGA GTGATTGAGA ATGCACTCTC AAAGACAGTG   180
GATATGCGTG AAGTCTTAAG GCATGAATCT GTTGTACTCT CCCCGAATGT AATGGACGAG   240
GGAGCAATAG ACGAGCTGAT TCGTGCCTTT GGGGAGTCGG GCATAGCTGA AAATGTGCAG   300
TTTGATGTTG CAATAGACAT TGCTCGCCAC TGTTCTGATG TGGGGAGCTC TCAGAGGTCA   360
ACCCTTATTG GTAAAAGCCC CTTCTGTGAG TTAAATAGGT CTGAAATTGC CGGAATAATA   420
AGGGAGGTGA CCACGCTGCG CAGATTTTGC ATGTACTACG CAAAGATTGT GTGGAACATC   480
CATTTGGAGA CGGGAATACC ACCAGCTAAT TGGGCCAAGA AAGGATTTAA TGAGAATGAA   540
AAGTTTGCAG CCTTTGACTT CTTCCTTGGA GTCACAGATG AAAGCGCGCT TGAGCCTAAG   600
GGTGGAGTCA AGAGAGCTCC AACAAAAGCA G                                  631
```

The polypeptide has a deduced amino acid sequence corresponding to SEQ. ID. No. 22 as follows:

```
Met Ala Ser Gln Val Gly Lys Leu Pro Gly Glu Ser Asn Glu Ala Tyr
1               5                   10                  15

Glu Ala Arg Leu Lys Ala Leu Glu Leu Ala Arg Ala Gln Lys Ala Pro
            20                  25                  30

Glu Val Ser Asn Gln Pro Pro Thr Leu Gly Gly Ile Leu Ala Lys Arg
        35                  40                  45

Lys Arg Val Ile Glu Asn Ala Leu Ser Lys Thr Val Asp Met Arg Glu
    50                  55                  60

Val Leu Arg His Glu Ser Val Val Leu Ser Pro Asn Val Met Asp Glu
65                  70                  75                  80

Gly Ala Ile Asp Glu Leu Ile Arg Ala Phe Gly Glu Ser Gly Ile Ala
                85                  90                  95

Glu Asn Val Gln Phe Asp Val Ala Ile Asp Ile Ala Arg His Cys Ser
            100                 105                 110
```

```
Asp Val Gly Ser Ser Gln Arg Ser Thr Leu Ile Gly Lys Ser Pro Phe
        115                 120                 125

Cys Glu Leu Asn Arg Ser Glu Ile Ala Gly Ile Ile Arg Glu Val Thr
    130                 135                 140

Thr Leu Arg Arg Phe Cys Met Tyr Tyr Ala Lys Ile Val Trp Asn Ile
145                 150                 155                 160

His Leu Glu Thr Gly Ile Pro Pro Ala Asn Trp Ala Lys Lys Gly Phe
                165                 170                 175

Asn Glu Asn Glu Lys Phe Ala Ala Phe Asp Phe Phe Leu Gly Val Thr
            180                 185                 190

Asp Glu Ser Ala Leu Glu Pro Lys Gly Gly Val Lys Arg Ala Pro Thr
        195                 200                 205

Lys Ala
    210
```

The DNA molecule which constitutes a substantial portion of the RSPaV strain RSP158 genome comprises the nucleotide sequence corresponding to SEQ. ID. No. 23 as follows:

```
GAAGCTAGCA CATTTCTGTT CAACACTATG GCTAACATGT TGTTCACTTT TCTGAGATAT     60
GAACTGACGG GTTCAGAGTC AATAGCATTT GCAGGGGATG ATATGTGTGC TAATAGAAGG    120
TTGCGGCTTA AAACGGAGCA TGAGGGTTTT CTGAACATGA TCTGCCTTAA GGCCAAGGTT    180
CAGTTTGTTT CCAACCCCAC ATTCTGTGGA TGGTGCTTAT TTAAGGAGGG AATCTTCAAG    240
AAACCTCAAC TAATTTGGGA GCGAATATGC ATAGCCAGAG AGATGGGCAA TCTGGAGAAC    300
TGTATTGACA ATTATGCGAT AGAAGTGTCC TATGCATATA GATTGGGTGA GCTATCAATT    360
GAAATGATGA CAGAAGAAGA AGTGGAGGCA CACTACAATT GTGTGAGGTT CCTGGTTAGG    420
AACAAGCATA AGATGAGGTG CTCAATTTCA GGCCTGTTTG AAGTGGTTGA TTAGGCCTTA    480
AGTATTTGGC GTTGTTCGAG TTATTATGAA TAATTTAGTT AAAGCATTAT CAGCCTTCGA    540
GTTTATAGGT GTTTTCAATG TGCTCAAATT TCCAGTTGTT ATACATAGTG TGCCTGGTAG    600
TGGTAAGAGT AGCTTAATAA GGGAATTAAT CTCAGAGGAC GAGAGTTTCG TGGCTTTCAC    660
AGCAGGTGTT CCAGACAGTC CTAACCTCAC AGGGAGGTAC ATCAAGCCTT ACTCACCAGG    720
ATGCGCAGTG CAAGGAAAAG TGAATATACT TGATGAGTAC TTGTCCGTTC AAGACATTTC    780
GGGTTTTGAT GTACTGTTTT CAGACCCGTA CCAGAATATC AGTATTCCCC AAGAGGCGCA    840
TTTCATTAAG TCCAAGACTT GTAGGTTTGG TGTGAACACT TGCAAATACC TTTCCTCTTT    900
CGGTTTCGAA GTTAGCAGCG ACGGGCTGGA CGACGTCATT GTGGGATCGC CCTTCACTCT    960
```

```
AGATGTTGAA GGGGTGCTGA TATGTTTTGG CAAGGAGGCG GTAGATCTCG CTGTTGCGCA    1020
CAACTCTGAA TTCAAGTTGC CGTGTGAGGT TCGAGGTTCA ACCTTCAATG TGGTAACCCT    1080
TTTGAAATCA AGAGACCCAA CCCCAGAGGA CAGGCACTGG TTTTACATCG CTGCCACAAG    1140
ACATAGGAAG AAATTGGTCA TTATGCAGTA AAATGCCTTT TCAGCAGCCT GCTAATTGGG    1200
CAAAAACCAT AACTCCATTG ACTATTGGCT TAGGAATTGG ACTTGTGCTG CATTTTCTGA    1260
GAAAGTCAAA TCTACCATAT TCAGGAGACA ACATCCATCA ATTTCCTCAC GGGGGGCGTT    1320
ACCGGGACGG CACAAAAAGT ATAACTTACT GTGGCCCTAA GCAGTCCTTC CCCAGTTCAG    1380
GAATATTTGG TCAGTCTGAG AATTTTGTGC CCTTAATGCT TGTCATAGGT CTAATTGCAT    1440
TCATACATGT ATTGTCTGTT TGGAATTCTG GTCTTGGTAG GAATTGCAAT TGCCATCCAA    1500
ATCCTTGCTC ATGTAGACAA CAGTAGTGGC AGTCACCAAG GTTGCTTTAT CAGGGCCACT    1560
GGAGAGTCTA TTTTGATTGA AAATTGTGGC CCAAGCGAGG CCCTTGCATC AACAGTGAGG    1620
GAGGTGTTGG GGGGTTTGAA GGCTTTAGGA ATTAGCCATA CTACTGAAGA AATTGATTAT    1680
CGTTGTTAAA TTGGTTAAAT GGCGAGTCAA GTTGGTAAGC TCCCCGGAGA ATCAAATGAG    1740
GCATTTGAAG CCCGGCTGAA ATCACTGGAG TTGGCTAGAG CTCAAAAGCA GCCAGAAGGT    1800
TCAAACACAC CGCCTACTCT CAGTGGTGTG CTTGCCAAAC GTAAGAGGGT TATTGAGAAT    1860
GCACTCTCAA AGACAGTGGA CATGAGGGAG GTGTTGAAAC ACGAAACGGT TGTAATTTCC    1920
CCAAATGTCA TGGATGAGGG TGCAATAGAT GAACTGATTC GTGCATTCGG AGAATCAGGC    1980
ATAGCTGAGA GCGCACAATT TGATGTGGC                                      2009
```

The RSP158 strain contains five open reading frames (i.e., ORF1-5). ORF1 and ORF5 are only partially sequenced. The nucleotide sequence of RSP158 is 87.6% identical to the corresponding region of RSPaV-1 (type strain). The numbers of amino acid residues of corresponding ORFs of RSP158 and RSPaV-1 (type strain) are exactly the same. In addition, the amino acid sequences of these ORFs have high identities to those of RSPaV-1: 99.3% for ORF1, 95% for ORF2, 99.1% for ORF3, 88.8% for ORF4, and 95.1% for ORF5. The nucleotide and amino acid sequence information of the RSP158 ORFs are described below.

Another DNA molecule of the present invention (RSP158 incomplete ORF1) includes nucleotides 1-447 of SEQ. ID. No. 23. This DNA molecule is believed to code for a polypeptide portion of a RSP158 replicase and comprises a nucleotide sequence corresponding to SEQ. ID. No. 24 as follows:

```
GAAGCTAGCA CATTTCTGTT CAACACTATG GCTAACATGT TGTTCACTTT TCTGAGATAT    60
GAACTGACGG GTTCAGAGTC AATAGCATTT GCAGGGGATG ATATGTGTGC TAATAGAAGG   120
TTGCGGCTTA AAACGGAGCA TGAGGGTTTT CTGAACATGA TCTGCCTTAA GGCCAAGGTT   180
CAGTTTGTTT CCAACCCCAC ATTCTGTGGA TGGTGCTTAT TTAAGGAGGG AATCTTCAAG   240
AAACCTCAAC TAATTTGGGA GCGAATATGC ATAGCCAGAG AGATGGGCAA TCTGGAGAAC   300
TGTATTGACA ATTATGCGAT AGAAGTGTCC TATGCATATA GATTGGGTGA GCTATCAATT   360
GAAATGATGA CAGAAGAAGA AGTGGAGGCA CACTACAATT GTGTGAGGTT CCTGGTTAGG   420
AACAAGCATA AGATGAGGTG CTCAATT                                       447
```

The polypeptide encoded by the nucleotide sequence of SEQ. ID. No. 24 has a deduced amino acid sequence corresponding to SEQ. ID. No. 25 as follows:

```
Glu Ala Ser Thr Phe Leu Phe Asn Thr Met Ala Asn Met Leu Phe Thr
1               5                   10                  15

Phe Leu Arg Tyr Glu Leu Thr Gly Ser Glu Ser Ile Ala Phe Ala Gly
            20                  25                  30

Asp Asp Met Cys Ala Asn Arg Arg Leu Arg Leu Lys Thr Glu His Glu
        35                  40                  45

Gly Phe Leu Asn Met Ile Cys Leu Lys Ala Lys Val Gln Phe Val Ser
    50                  55                  60

Asn Pro Thr Phe Cys Gly Trp Cys Leu Phe Lys Glu Gly Ile Phe Lys
65                  70                  75                  80

Lys Pro Gln Leu Ile Trp Glu Arg Ile Cys Ile Ala Arg Glu Met Gly
                85                  90                  95

Asn Leu Glu Asn Cys Ile Asp Asn Tyr Ala Ile Glu Val Ser Tyr Ala
            100                 105                 110

Tyr Arg Leu Gly Glu Leu Ser Ile Glu Met Met Thr Glu Glu Glu Val
        115                 120                 125

Glu Ala His Tyr Asn Cys Val Arg Phe Leu Val Arg Asn Lys His Lys
    130                 135                 140

Met Arg Cys Ser Ile
145
```

Another DNA molecule of the present invention (RSP158 ORF2) includes nucleotides 506-1171 of SEQ. ID. No. 23. This DNA molecule codes for a first protein or polypeptide of the RSP158 triple gene block and comprises a nucleotide sequence corresponding to SEQ. ID. No. 26 as follows:

```
ATGAATAATT TAGTTAAAGC ATTATCAGCC TTCGAGTTTA TAGGTGTTTT CAATGTGCTC    60
AAATTTCCAG TTGTTATACA TAGTGTGCCT GGTAGTGGTA AGAGTAGCTT AATAAGGGAA   120
TTAATCTCAG AGGACGAGAG TTTCGTGGCT TTCACAGCAG GTGTTCCAGA CAGTCCTAAC   180
CTCACAGGGA GGTACATCAA GCCTTACTCA CCAGGATGCG CAGTGCAAGG AAAAGTGAAT   240
ATACTTGATG AGTACTTGTC CGTTCAAGAC ATTTCGGGTT TTGATGTACT GTTTTCAGAC   300
CCGTACCAGA ATATCAGTAT TCCCCAAGAG GCGCATTTCA TTAAGTCCAA GACTTGTAGG   360
TTTGGTGTGA ACACTTGCAA ATACCTTTCC TCTTTCGGTT TCGAAGTTAG CAGCGACGGG   420
CTGGACGACG TCATTGTGGG ATCGCCCTTC ACTCTAGATG TTGAAGGGGT GCTGATATGT   480
TTTGGCAAGG AGGCGGTAGA TCTCGCTGTT GCGCACAACT CTGAATTCAA GTTGCCGTGT   540
GAGGTTCGAG GTTCAACCTT CAATGTGGTA ACCCTTTTGA AATCAAGAGA CCCAACCCCA   600
GAGGACAGGC ACTGGTTTTA CATCGCTGCC ACAAGACATA GGAAGAAATT GGTCATTATG   660
CAGTAA                                                              666
```

The first protein or polypeptide of the RSP158 triple gene block has a deduced amino acid sequence corresponding to SEQ. ID. No. 27 as follows:

```
Met Asn Asn Leu Val Lys Ala Leu Ser Ala Phe Glu Phe Ile Gly Val
1               5                   10                  15

Phe Asn Val Leu Lys Phe Pro Val Val Ile His Ser Val Pro Gly Ser
            20                  25                  30

Gly Lys Ser Ser Leu Ile Arg Glu Leu Ile Ser Glu Asp Glu Ser Phe
        35                  40                  45

Val Ala Phe Thr Ala Gly Val Pro Asp Ser Pro Asn Leu Thr Gly Arg
    50                  55                  60

Tyr Ile Lys Pro Tyr Ser Pro Gly Cys Ala Val Gln Gly Lys Val Asn
65                  70                  75                  80

Ile Leu Asp Glu Tyr Leu Ser Val Gln Asp Ile Ser Gly Phe Asp Val
                85                  90                  95

Leu Phe Ser Asp Pro Tyr Gln Asn Ile Ser Ile Pro Gln Glu Ala His
            100                 105                 110

Phe Ile Lys Ser Lys Thr Cys Arg Phe Gly Val Asn Thr Cys Lys Tyr
        115                 120                 125

Leu Ser Ser Phe Gly Phe Glu Val Ser Ser Asp Gly Leu Asp Asp Val
    130                 135                 140

Ile Val Gly Ser Pro Phe Thr Leu Asp Val Glu Gly Val Leu Ile Cys
145                 150                 155                 160
```

```
Phe Gly Lys Glu Ala Val Asp Leu Ala Val Ala His Asn Ser Glu Phe
                165                 170                 175

Lys Leu Pro Cys Glu Val Arg Gly Ser Thr Phe Asn Val Val Thr Leu
            180                 185                 190

Leu Lys Ser Arg Asp Pro Thr Pro Glu Asp Arg His Trp Phe Tyr Ile
        195                 200                 205

Ala Ala Thr Arg His Arg Lys Lys Leu Val Ile Met Gln
    210                 215                 220
```

The first protein or polypeptide of the RSP158 triple gene block has a molecular weight of about 20 to 26 kDa., preferably 24.4 kDa.

Another DNA molecule of the present invention (RSP158 ORF3) includes nucleotides 1173-1526 of SEQ. ID. No. 23. This DNA molecule codes for a second protein or polypeptide of the RSP158 triple gene block and comprises a nucleotide sequence corresponding to SEQ. ID. No. 28 as follows:

```
ATGCCTTTTC AGCAGCCTGC TAATTGGGCA AAAACCATAA CTCCATTGAC TATTGGCTTA     60

GGAATTGGAC TTGTGCTGCA TTTTCTGAGA AAGTCAAATC TACCATATTC AGGAGACAAC    120

ATCCATCAAT TTCCTCACGG GGGGCGTTAC CGGGACGGCA CAAAAAGTAT AACTTACTGT    180

GGCCCTAAGC AGTCCTTCCC CAGTTCAGGA ATATTTGGTC AGTCTGAGAA TTTTGTGCCC    240

TTAATGCTTG TCATAGGTCT AATTGCATTC ATACATGTAT TGTCTGTTTG GAATTCTGGT    300

CTTGGTAGGA ATTGCAATTG CCATCCAAAT CCTTGCTCAT GTAGACAACA GTAG          354
```

The second protein or polypeptide of the RSP158 triple gene block has a deduced amino acid sequence corresponding to SEQ. ID. No. 29 as follows:

```
Met Pro Phe Gln Gln Pro Ala Asn Trp Ala Lys Thr Ile Thr Pro Leu
1               5                   10                  15

Thr Ile Gly Leu Gly Ile Gly Leu Val Leu His Phe Leu Arg Lys Ser
            20                  25                  30

Asn Leu Pro Tyr Ser Gly Asp Asn Ile His Gln Phe Pro His Gly Gly
        35                  40                  45

Arg Tyr Arg Asp Gly Thr Lys Ile Thr Tyr Cys Gly Pro Lys Gln Ser
    50                  55                  60

Phe Pro Ser Ser Gly Ile Phe Gly Gln Ser Glu Asn Phe Val Pro Leu
65                  70                  75                  80

Met Leu Val Ile Gly Leu Ile Ala Phe Ile His Val Leu Ser Val Trp
                85                  90                  95
```

```
Asn Ser Gly Leu Gly Arg Asn Cys Asn Cys His Pro Asn Pro Cys Ser
            100                 105                 110

Cys Arg Gln Gln
        115
```

The second protein or polypeptide of the RSP158 triple gene block has a molecular weight of about 10 to 15 kDa., preferably 12.9 kDa.

Another DNA molecule of the present invention (RSP158 ORF4) includes nucleotides 1447-1689 of SEQ. ID. No. 23. This DNA molecule codes for a third protein or polypeptide of the RSP158 triple gene block and comprises a nucleotide sequence corresponding to SEQ. ID. No. 30 as follows:

```
ATGTATTGTC TGTTTGGAAT TCTGGTCTTG GTAGGAATTG CAATTGCCAT CCAAATCCTT     60

GCTCATGTAG ACAACAGTAG TGGCAGTCAC CAAGGTTGCT TTATCAGGGC CACTGGAGAG    120

TCTATTTTGA TTGAAAATTG TGGCCCAAGC GAGGCCCTTG CATCAACAGT GAGGGAGGTG    180

TTGGGGGGTT TGAAGGCTTT AGGAATTAGC CATACTACTG AAGAAATTGA TTATCGTTGT    240

TAA                                                                  243
```

The third protein or polypeptide of the RSP158 triple gene block has a deduced amino acid sequence corresponding to SEQ. ID. No. 31 as follows:

```
Met Tyr Cys Leu Phe Gly Ile Leu Val Leu Val Gly Ile Ala Ile Ala
1               5                   10                  15

Ile Gln Ile Leu Ala His Val Asp Asn Ser Ser Gly Ser His Gln Gly
            20                  25                  30

Cys Phe Ile Arg Ala Thr Gly Glu Ser Ile Leu Ile Glu Asn Cys Gly
        35                  40                  45

Pro Ser Glu Ala Leu Ala Ser Thr Val Arg Glu Val Leu Gly Gly Leu
    50                  55                  60

Lys Ala Leu Gly Ile Ser His Thr Thr Glu Glu Ile Asp Tyr Arg Cys
65                  70                  75                  80
```

The third protein or polypeptide of the RSP158 triple gene block has a molecular weight of about 5 to 10 kDa., preferably 8.4 kDa.

Yet another DNA molecule of the present invention (RSP158 ORF5) includes nucleotides 1699-2009 of SEQ. ID. No. 23. This DNA molecule codes for a partial RSP158 coat protein or polypeptide and comprises a nucleotide sequence corresponding to SEQ. ID. No. 32 as follows:

```
ATGGCGAGTC AAGTTGGTAA GCTCCCCGGA GAATCAAATG AGGCATTTGA AGCCCGGCTG     60
AAATCACTGG AGTTGGCTAG AGCTCAAAAG CAGCCAGAAG GTTCAAACAC ACCGCCTACT    120
CTCAGTGGTG TGCTTGCCAA ACGTAAGAGG GTTATTGAGA ATGCACTCTC AAAGACAGTG    180
GACATGAGGG AGGTGTTGAA ACACGAAACG GTTGTAATTT CCCCAAATGT CATGGATGAG    240
GGTGCAATAG ATGAACTGAT TCGTGCATTC GGAGAATCAG GCATAGCTGA GAGCGCACAA    300
TTTGATGTGG C                                                         311
```

The polypeptide has a deduced amino acid sequence corresponding to SEQ. ID. No. 33 as follows:

```
Met Ala Ser Gln Val Gly Lys Leu Pro Gly Glu Ser Asn Glu Ala Phe
1               5                   10                  15

Glu Ala Arg Leu Lys Ser Leu Glu Leu Ala Arg Ala Gln Lys Gln Pro
            20                  25                  30

Glu Gly Ser Asn Thr Pro Pro Thr Leu Ser Gly Val Leu Ala Lys Arg
        35                  40                  45

Lys Arg Val Ile Glu Asn Ala Leu Ser Lys Thr Val Asp Met Arg Glu
    50                  55                  60

Val Leu Lys His Glu Thr Val Val Ile Ser Pro Asn Val Met Asp Glu
65                  70                  75                  80

Gly Ala Ile Asp Glu Leu Ile Arg Ala Phe Gly Glu Ser Gly Ile Ala
                85                  90                  95

Glu Ser Ala Gln Phe Asp Val
            100
```

The following seven cDNA clones are located at the central part of the ORF1 of RSPaV-1 and all have high identities (83.6- 98.4%) in nucleotide sequence with the comparable regions of RSPaV-1. When their nucleotide sequences are aligned with MegAlign (DNAStar), a highly conserved region of ca. 600 nucleotides was found. The universal primers BM98-3F/BM98-3R (SEQ. ID. Nos. 51 and 52, *infra*) were designed based on the conserved nucleotide sequences of this region.

Portions of the genome from yet other strains of *Rupestris* stem pitting associated viruses have also been isolated and sequenced. These include strains designated 140/94-19 (T7+R1), 140/94-24 (T7+R1), 140/94-2 (T3+F1), 140/94+42 (T7+R1), 140/94-64 (T7+R1), 140-94-72 (T7+R1), and 140/94-6 (T3+BM98-3F+F2).

The nucleotide sequence of 140/94-19 (T7+R1) corresponds to SEQ. ID. No. 34 as follows:

```
GCAGGATTGA AGGCTGGCCA CTGTGTGATT TTTGATGAGG TCCAGTTGTT TCCTCCTGGA    60
TACATCGATC TATGCTTGCT TATTATACGT AGTGATGCTT TCATTTCACT TGCCGGTGAT   120
CCATGTCAAA GCACATATGA TTCGCAAAAG GATCGGGCAA TTTTGGGCGC TGAGCAGAGT   180
GACATACTTA GAATGCTTGA GGGCAAAACG TATAGGTATA ACATAGAAAG CAGGAGGTTT   240
GTGAACCCAA TGTTCGAATC AAGACTGCCA TGTCACTTCA AAAAGGGTTC GATGACTGCC   300
GCTTTCGCTG ATTATGCAAT CTTCCATAAT ATGCATGACT TTCTCCTGGC GAGGTCAAAA   360
GGTCCTTTGG ATGCCGTTTT GGTTTCCAGT TTTGAGGAGA AAAAGATAGT CCAGTCCTAC   420
TTTGGAATGA AACAGCTCAC ACTCACATTT GGTGAATCAA CTGGGTTGAA TTTCAAAAAT   480
GGGGGAATTC TCATATCACA TGATTCCTTT CACACAGATG ATCGGCCGGT GGCTTACTGC   540
TTTATCTCGC TTCAGCCACA ATTTGGATTT GGTGAACATT ACAGGTCTGA GGGTGGAAAG   600
TTTCCTCTCG CACTTTGCTG GCAAACCCCT CTACCATTTT TTAACAGCCA AAAGTGGGGA   660
GAATGTCATA CGAGATTTGC TCCCAGGTGA GCCTAACTTC TTCAGTGGCT TTAACGTTAG   720
CATTGGAAAG AATGAAGGTG TTAGGGAGGA GAAGTTATGT GGTGACCCAT GGTTAAAAGT   780
CATGCTTTTC CTGGGTCAAG ATGAGGATTG TGAAGTTGAA GAGATGGAGT CAGAGTGCTC   840
AAATGAAGAA TGGTTTAAAA CCCACATTCC CCTGAGTAAT CTGGAGTCAA CCAGGGCTAG   900
GTGGGTGGGT AAAATGGCTT TGAAAGAGTA TCGGGAGGTG CGTTGTGGTT ATGAAATGAC   960
TCAACAATTC TTTGATGAGC ATAGGGGTGG AACTGGTGAG CAACTGAGCA ATGCATGTGA  1020
GAGGTTTGAA AGCATTTACC CAAGGCATAA AGGAAATGAT TCAATAACCT TCCTTATGGC  1080
TGTCCGAAAG CGTCTCAAAT TTTCGAAGCC CCAGGTTGAA GCTGCCAAAC TGAGGCGGGC  1140
CAAACCATAT GGGAAATTCT TATTAGACTT TCCTATCCAA AATCCCATTG AAAGCCAGTC  1200
ATAATT                                                             1206
```

The nucleotide sequence of 140/94-24 (T7+R1) corresponds to SEQ. ID. No. 35 as follows:

```
ATTAACCCAA ATGGTAAGAT TTCCGCCTTG TTTGATATAA CCAATGAGCA CATAAGGCAT    60
GTTGAGAAGA TCGGCAATGG CCCTCAGAGC ATAAAAGTAG ATGAGTTGAG GAAGGTTAAG   120
CGATCCGCCC TTGATCTTCT TTCAATGAAT GGGTCCAAAA TAACCTATTT TCCAAACTTT   180
GAGCGGGCTG AAAAGTTGCA AGGGTGCTTG CTAGGGGGCC TAACTGGTGT CATAAGTGAT   240
```

```
GAAAAGTTCA GTGATGCAAA ACCCTGGCTT TCTGGTATAT CAACTGCGGA TATAAAGCCA      300
AGAGAGCTAA CTGTCGTGCT TGGCACTTTT GGGGCTGGAA AGAGTTTCTT GTATAAGAGT      360
TTCATGAAGA GATCTGAGGG AAAATTTGTA ACTTTTGTTT CCCCTAGACG AGCCTTGGCA      420
AATTCAATCA AAAATGATCT TGAAATGGAT GATGGCTGCA AAGTTGCCAA AGCAGGCAAA      480
TCAAAGAAGG AAGGGTGGGA TGTAGTGACC TTTGAAGTTT TCCTTAGAAA AGTTTCTGGT      540
TTGAAAGCTG GTCATTGTGT GATTTTTGAT GAGGTTCAGT TGTTTCCCCC TGGATACATC      600
GATCTGTGTT TACTTGTCAT ACGAAGTGAT GCTTTCATTT CACTTGCTGG TGATCCATGC      660
CAGAGCACAT ATGATTCACA GAAGGATCGA GCAATTTTGG GAGCTGAGCA GAGTGACATA      720
CTCAGACTGC TTGAAGGAAA GACATATAGG TACAACATAG AAAGCAGACG TTTTGTGAAC      780
CCAATGTTTG AATCTAGACT ACCATGTCAC TTCAAAAAGG GTTCAATGAC TGCAGCCTTT      840
GCTGATTATG CAATCTTCCA CAATATGCAT GACTTCCTCC TGGCGAGGTC AAAAGGCCCC      900
TTGGATGCTG TTCTAGTTTC CAGTTTTGAG GAGAAGAAAA TAGTCCAATC CTACTTTGGG      960
ATGAAGCAAC TCACTCTCAC ATTTGGTGAA TCAACTGGGT TGAACTTCAA AAATGGAGGA     1020
ATTCTCATAT CACATGACTC CTTTCATACT GACGATCGAC GGTGGCTTAC TGCTTTATCT     1080
CGATTCAGCC ATAATTTGGA TTTGGTGAAC ATCACAGGTC TTGAGGGTGG AAAGTTTTCT     1140
CTCACATTTT GCTGGTAAAC CCCTTTACCA CTTTTTGACG GCTTAAAAGT GGAGAGAATG     1200
TCATACGAGA CCTGCTTCAG GTGAGCCTAA CTTCTTTTAG GGGTTCAATG TCAGCATTGG     1260
AAAAAAATGG AAGGGGTTAG AGAA                                            1284
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The nucleotide sequence of 140/94-2 (T3+F1) corresponds to SEQ. ID. No. 36 as follows:

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CATTTTTAAA ATTTAATCCA GTCGACTCAC CAAATGTGAG CGTAAGCTGT TTCATCCCAA       60
AGTAGGACTG GACTATTTTC TTCTCCTCAA AACTAGAAAC CAGAATGGCA TCCAAAGGAC      120
CTTTTGACCT TGCCAGGAGG AAATCATGCA TATTGTGGAA AATGGCATAA TCAGCAAAGG      180
CAGCAGTCAT TGTACCCTTT TTGAAGTGAC ATGGCAGTCG AGATTCAAAC ATTGGGTTCA      240
CAAATCTTCT GCTTTCTATG TTGTACCTAT ACGTCTTGCC TTCAAGTATT TTGAGTATGT      300
CACTCTGCTC AGCGCCCAAA ATCGCCCGAT CTTTTTGTGA GTCATATGTG CTCTGACATG      360
GGTCACCAGC AAGTGAAATG AAAGCATCAC TACGTATAAT AAGCAAACAT AGATCGATGT      420
ATCCAGGGGG AAACAACTGG ACCTCATCGA AAATTACACA GTGACCAGCT TTTAGACCTG      480
CAACTTTTCT AAGGAAGACT TCAAAAGTCA CAACATCCCA TCCTTCCTTC TTTGACCTGC      540
CTGCTTTGGC AACTTTGCAG CTATCATCCA TTTCAAGATC ATTTTTGATT GAATTCGCTA      600
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GAGCCCGTCT GGGGGAAACA AAAGTTACGA ATTTACCCTC AGATCTTTTC ATAAAGCTCT 660
TGTACAAAAA GCTTTTTCCG GCTCCAAATG TGCCAAGCAC AACAGTTAGC TCCCTCGGCT 720
TAATGTCAGT AGTTGATATA CCAGAAAGCC AGGGCTTTGC ATCACTGAAC TTCTCATCAC 780
TTATGACACC AGTTAGGCCT CCTAGCAGAC ACCCTTGCAA CTTTTCAGCC CGCTCAAAAC 840
TTGGGAAGTA GGTTACCTTG GACCCATTAA TTGAAAGAAG ATCAAGGGCG GATCGCTTGA 900
CCTTTCGCAA TTCATCTACT TTAATGCTCT GAGGGCCATT ACCTATCTTT TCAACATGCC 960
TTATGTGCTC ATTAGTTATG TCAAACAGAG CGGAAAACTT GCCATGTGGA TTAATCACCT 1020
CAATTTCCCC ATTTATGTCA CACTTAGCGC AAATGTCAAA AGCCTCAAAG GCTTCAGCTA 1080
AGTTACATCA TGTTGAGCCT CCCCCTTGGC AAAGCTCCTC AAAAATGTGG TTAGTGCTAG 1140
GCCTGCACAA TAATTAACAC ATCAACTTCA CCCTGCCAAT GCTGAACAAT ACTGTTATCA 1200
TGCAACCATC CATGGGGCAC ATGGTTGGAA TTGATTGATT TAAGGCAAAA ATCCCCACAG 1260
GGGGCATCCC CTTCCCCAAT TTCCACTGAT TCATACTCTG GCGTTATCAT ATCAACCCAA 1320
TGTGTCAAAT ACAAATAATG CAATCTCTCA TCTCCGATAA CATTTCCCCC ATTTTTTAAA 1380
AATGGTGGGG TGAAAATTGG AA 1402

The nucleotide sequence of 140/94-42 (T7+R1) corresponds to SEQ. ID. No. 37 as follows:

GTGGTTTTTG CAACAACAGG CCCAGGTCTA TCTAAGGTTT TGGAAATGCC TCGAAGCAAG 60
AAGCAATCTA TTCTGGTTCT TGAGGGAGCC CTATCCATAG AAACGGACTA TGGCCCAAAA 120
GTTCTGGGAT CTTTTGAAGT TTTCAAAGGG GATTTCAACA TTAAAAAAAT GGAAGAAAGT 180
TCCATCTTTG TAATAACATA CAAGGCCCCA GTTAGATCTA CTGGCAAGTT GAGGGTCCAC 240
CAATCAGAAT GCTCATTTTC TGGATCCAAG GAGGTATTGC TGGGTTGTCA GATTGAGGCA 300
TGTGCTGATT ATGATATTGA TGATTTCAAT ACTTTCTTTG TACCTGGTGA TGGTAATTGC 360
TTTTGGCATT CAGTTGGTTT CTTACTCAGT ACTGACGGAC TTGCTTTGAA GGCCGGCATT 420
CGTTCTTTCG TGGAGAGTGA ACGCCTGGTG AGTCCAGATC TTTCAGCCCC AACCATTTCT 480
AAACAACTGG GGGAAAATGC TTATGCCGAG AATGAGATGA TTGCATTATT TTGTATTCGA 540
CACCATGTGA GGCTGATAGT GATTACGCCA GAGTATGAAG TCAGTTGGAA ATTTGGGGAA 600
GGTGAATGGC CCCTGTGCGG AATTCTTTGC CTTAAATCAA ATCACTTCCA ACCATGTGCC 660
CCATTGAATG GTTGCATGAT TACAGCTATT GCTTCAGCAC TTGGTAGGCG TGAAGTTGAT 720
GTGCTTAATT ATCTGTGCAG GCCTAGCACT AACCACATTT TTGAGGAGCT TTGCCAAGGG 780

GGAGGCCTCA ACATGATGTA CTTAGCTGAA GCCTTTGAGG CTTTTGACAT TTGCGCTAAG 840
TGTGACATAA ATGGGGAAAT TGAGGTGATT AATCCACATG GCAAGTTTTC CGCTCTGTTT 900
GACATAACTA ATGAGCACAT AAGGCATGTT GAAAAGATAG GTAATGGCCC TCAGAGCATT 960
AAAGTAGATG AATTGCGAAA GGTCAAGCGA TCTGCCCTTG ATCTTCTTTC AATTAATGGG 1020
TCCAAGGTAA CCTACTTCCC AAGTTTTGAG CGGGCTGAAA AGTTGCAAGG GTGTCTGCTA 1080
GGAGGCCTAA CTGGTGTCAT AAGTGATGAG AAAGTCAGTG ATGCAAAGCC CTGCTTTTTG 1140
GTATATCAAC TACTGACATT AAGCCGAGGG AGCTAACTGT TGTGCTTTGG CACATTTGGA 1200
GCCCGGAAAA AGCCTTTTGT ACCAAGAGCT TTATTG 1236

The nucleotide sequence of 140/94-6 (T3 + BM98 – 3F + F2) corresponds to SEQ. ID. No. 38 as follows:

GTCTAACTGG CGTTATAAGT GATGAGAAAT TCAGTGATGC AAAACCTTGG CTTTCTGGTA 60
TATCTACTAC AGATATTAAG CCAAGGGAAT TAACTGTTGT GCTTGGTACA TTTGGGGCTG 120
GGAAGAGTTT CTTGTACAAG AGTTTCATGA AAAGGTCTGA GGGTAAATTC GTAACCTTTG 180
TTTCTCCCAG ACGTGCTTTA GCAAATTCAA TCAAAAATGA TCTTGAAATG GATGATAGCT 240
GCAAAGTTGC CAAAGCAGGT AGGTCAAAGA AGGAAGGGTG GGATGTAGTA ACTTTTGAGG 300
TCTTCCTCAG AAAAGTTGCA GGATTGAAGG CTGGCCACTG TGTGATTTTT GATGAGGTCC 360
AGTTGTTTCC TCCTGGATAC ATCGATCTAT GCTTGCTTAT TATACGTAGT GATGCTTTCA 420
TTTCACTTGC CGGTGATCCA TGTCAAAGCA CATATGATTC GCAAAAGGAT CGGGCAATTT 480
TGGGCGCTGA GCAGAGTGAC ATACTTAGAA TGCTTGAGGG CAAAACGTAT AGGTATAACA 540
TAGAAAGCAG GAGGTTTGTG AACCCAATGT TCGAATCAAG ACTGCCATGT CACTTCAAAA 600
AGGGTTCGAT GACTGCCGCT TTCGCTGATT ATGCAATCTT CCATAATATG CATGACTTTC 660
TCCTGGCGAG GTCAAAAGGT CCTTTGGATG CCGTTTTGGT TTCCAGTTTT GAGGAGAAAA 720
AGATAGTCCA GTCCTACTTT GGAATGAAAC AGCTCACACT CACATTTGGT GAATCAACTG 780
GGTTGAATTT CAAAAATGGG GGAATTCTCA TATCACATGA TTCCTTTCAC ACAGATGATC 840
GGCGGTGGCT TACTGCTTTA TCTCGCTTCA GCCACAATTT GGATTTGGTG AACATTACAG 900
GTCTGAGGTG GAAAGTTTCC TCTCGCACTT TGCTGGCAAA CCCCTCTACC ATTTTTTAAC 960
AGCCAAAAGT GGGGAGAATG TCATACGAGA TTTGCTCCCA GGTGAGCCTA ACTTCTTCAG 1020
TGGCTTTAAC GTTAGCATTG GAAAGAATGA AGGTGTTAGG GAGGAGAAGT TATGTGGTGA 1080
CCCATGGTTA AAAGTCATGC TTTTCCTGGG TCAAGATGAG GATTGTGAAG TTGAAGAGAT 1140
GGAGTCAGAG TGCTCAAATG AAGAATGGTT TAAAACCCAC ATTCCCCTGA GTAATCTGGA 1200

GTCAACCAGG GCTAGGTGGG TGGGTAAAAT GGCCTTGAAA GAGTATCGGG AGGTGCGTTG 1260

TGGTTATGAA ATGACTCAAC AATTCTTTGA TGACAT 1296

The nucleotide sequence of 140/94-64 (T7+R1) corresponds to SEQ. ID. No. 39 as follows:

ATGTTCACCA AATCCAAATT ATGGCTGAAG CGAGATAAAG CAGTAAGCCA CCGCCGATCA 60

TCTGTGTGAA AGGAATCATG TGATATGAGA ATTCCCCCAT TTTTGAAATT CAACCCAGTT 120

GATTCACCAA ATGTGAGTGT GAGCTGTTTC ATTCCAAAGT AGGACTGGAC TATCTTTTTC 180

TCCTCAAAAC TGGAAACCAA AACGGCATCC AAAGGACCTT TTGACCTCGC CAGGAGAAAG 240

TCATGCATAT TATGGAAGAT TGCATAATCA GCGAAAGCGG CAGTCATTGA GCCCTTTTTG 300

AATTGACATG GCAGTCTTGA TTCGAACATT GGATTCACAA ACCTCCTGCT TTCAATGTTA 360

TACCTATACG TCTTGCCCTC AAGCAGTCTA AGTATGTCAC TCTGCTCAGC GCCCAAAATT 420

GCCCGATCCT TTTGCGAATC ATATGTGCTT TGACATGGAT CACCGGCAAG TGAAATGAAA 480

GCATCACTAC GTATAATAAG CAAGCATAGA TCGATGTATC CAGGAGGAAA CAACTGGACC 540

TCATCGAAAA TCACACAGTG GCCAGCCTTC AATCCTGCAA CTTTTCTGAG GAAAACCTCA 600

AAAGTTACTA CATCCCACCC TTCCTTCTTT GACCTACCTG CTTTAGCAAC TTTGCAGCTA 660

TCATCCATTT CAAGATCATT TTTGATTGAA TTTGCTAAAG CACGTCTGGG AGAAACAAAG 720

GTTACGAATT TACCCTCAGA CCTTTTCATG AAACTCTTGT ACAAGAAACT CTTCCCAGCC 780

CCAAATGTAC CAAGCACGAC AGTCAACTCC CTTGGCTTAA TATCAGTAGT AGATATACCA 840

GAAAGCCAAG GTTTTGCATC ACTGAACTTC TCATCACTTA TAACGCCAGT TAGGCCCCCT 900

AGCAAAC 907

The nucleotide sequence of 140-94-72 (T7+R1) corresponds to SEQ. ID. No. 40 as follows:

AGAATGCTTA TGCTGAGAAT GAGATGATTG CATTATTTTG CATCCGGCAC CATGTAAGGC 60

TTATAGTAAT AACACCGGAA TATGAAGTTA GTTGGAAATT TGGGGAAAGT GAGTGGCCCC 120

TATGTGGAAT TCTTTGCCTG AGGTCCAATC ACTTCCAACC ATGCGCCCCG CTGAATGGTT 180

GCATGATCAC GGCTATTGCT TCAGCACTTG GGAGGCGTGA GGTTGATGTG TTAAATTATC 240

TGTGTAGGCC TAGCACTAAT CACATCTTTG AGGAGCTGTG CCAGGGCGGA GGGCTTAATA 300

TGATGTACTT GGCTGAAGCT TTTGAGGCCT TTGACATTTG TGCAAAGTGC GACATAAATG 360

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GGGAAATTGA GGTCATTAAC CCAAATGGCA AGATTTCCGC CTTGTTTGAT ATAACTAATG    420
AGCACATAAG GCATGTTGAG AAGATCAGCA ATGGCCCTCA GAGCATAAAA ATAGATGAGT    480
TGAGGAAGGT TAAGCGATCC CGCCTTGACC TTCTTTCAAT GAATGGGTCC AAAATAACCT    540
ATTTTCCAAA CTTTGAGCGG GCTGAAAAGT TGCAAGGGTG CTTGCTAGAG GGCCTGACTG    600
GTGTCATAAG TGATGAAAAG TTCAGTGATG CAAAACCTTG GCTTTCTGGT ATATCAACTG    660
CGGATATTAA GCCAAGAGAG CTAACTGTCG TGCTTGGCAC ATTTGGTGCT GGAAAGAGTT    720
TCTTGTATAA GAGTTTCATG AAGAGATCTG AAGGAAAATT TGTAACTTTT GTTTCCCCTA    780
GGCGAGCTTT GGCCAATTCG ATCAAGAATG ATCTTGAAAT GGATGATGGC TGCAAAGTTG    840
CCAAAGCAGG CAAGTCAAAG AAGGAAGGGT GGGATGTGGT AACATTTGAG GTTTTCCTTA    900
GAAAAGTTTC TGGTTTGAAG GCTGGTCATT GTGTGATTTT CGATGAGGTT CAGTTGTTTC    960
CCCCTGGATA TATCGATCTA TGTTTACTTG TCATACGCAG TGATGCTTTT ATTTCACTTG   1020
CCGGTGATCC ATGCCAGAGC ACATATGATT CACAAAAGGA TCGGGCAATT TTGGGAGCTG   1080
AGCAGAGTGA CATACTCAGA TTGCTTGAAG GAAAGACGTA TAGGTACAAC ATAGAAAGCA   1140
GACGTTTTGT GAACCCAATG TTTGAATTTA GACTACCATG TCACTTCAAA AAAGGGTTCA   1200
ATGACTGCTG CCTTTGCTGA TTATGCAATC TT
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Also encompassed by the present invention are fragments of the DNA molecules of the present invention. Suitable fragments capable of imparting RSP resistance to grape plants are constructed by using appropriate restriction sites, revealed by inspection of the DNA molecule's sequence, to: (i) insert an interposon (Felley et al., "Interposon Mutagenesis of Soil and Water Bacteria: A Family of DNA Fragments Designed for in vitro Insertion Mutagenesis of Gram-negative Bacteria," Gene, 52:147-15 (1987), which is hereby incorporated by reference) such that truncated forms of the RSP virus polypeptide or protein, that lack various amounts of the C-terminus, can be produced or (ii) delete various internal portions of the protein. Alternatively, the sequence can be used to amplify any portion of the coding region, such that it can be cloned into a vector supplying both transcription and translation start signals.

Suitable DNA molecules are those that hybridize to a DNA molecule comprising a nucleotide sequence of at least 15 continuous bases of SEQ. ID. No. 1 under stringent conditions characterized by a hybridization buffer comprising 0.9M sodium citrate ("SSC") buffer at a temperature of 37°C and remaining bound when

subject to washing with SSC buffer at 37°C; and preferably in a hybridization buffer comprising 20% formamide in 0.9M saline/0.9M SSC buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2x SSC buffer at 42°C.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of nucleotides that have minimal influence on the properties, secondary structure and hydropathic nature of the encoded protein or polypeptide. For example, the nucleotides encoding a protein or polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The nucleotide sequence may also be altered so that the encoded protein or polypeptide is conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The protein or polypeptide of the present invention is preferably produced in purified form (preferably, at least about 80%, more preferably 90%, pure) by conventional techniques. Typically, the protein or polypeptide of the present invention is isolated by lysing and sonication. After washing, the lysate pellet is re-suspended in buffer containing Tris-HCl. During dialysis, a precipitate forms from this protein solution. The solution is centrifuged, and the pellet is washed and re-suspended in the buffer containing Tris-HCl. Proteins are resolved by electrophoresis through an SDS 12% polyacrylamide gel.

The DNA molecule encoding the RSP virus protein or polypeptide of the present invention can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e., not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation

and replicated in unicellular cultures including procaryotic organisms and eukaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC184, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology, vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Suitable vectors are continually being developed and identified. Recombinant molecules can be introduced into cells via transformation, transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1982), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria or transformed via particle bombardment (i.e. biolistics). The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA ("mRNA") translation).

Transcription of DNA is dependent upon the presence of a promoter which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eukaryotic promoters differ from those of procaryotic promoters. Furthermore, eukaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promoters are not recognized and do not function in eukaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eukaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promoters vary in their "strength" (i.e., their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *rec*A promoter, ribosomal RNA promoter, the P_R and P_L promoters of coliphage lambda and others, including but not limited, to *lac*UV5, *omp*F, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lac*UV5 (*tac*) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operons, the addition of specific inducers is necessary for efficient transcription of the inserted

DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires a Shine-Dalgarno ("SD") sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecules encoding the various *Rupestris* stem pitting associated virus proteins or polypeptides, as described above, have been cloned into an expression system, they are ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The present invention also relates to RNA molecules which encode the various RSP virus proteins or polypeptides described above. The transcripts can be synthesized using the host cells of the present invention by any of the conventional techniques. The mRNA can be translated either *in vitro* or *in vivo*. Cell-free systems typically include wheat-germ or reticulocyte extracts. *In vivo* translation can be effected, for example, by microinjection into frog oocytes.

One aspect of the present invention involves using one or more of the above DNA molecules encoding the various proteins or polypeptides of a RSP virus to transform grape plants in order to impart RSP resistance to the plants. The mechanism by which resistance is imparted in not known. In one hypothetical mechanism, the transformed plant can express the coat protein or polypeptide, and,

when the transformed plant is inoculated by a RSP virus, such as RSPaV-1, the expressed coat protein or polypeptide surrounds the virus, thereby preventing translation of the viral DNA.

In this aspect of the present invention, the subject DNA molecule incorporated in the plant can be constitutively expressed. Alternatively, expression can be regulated by a promoter which is activated by the presence of RSP virus. Suitable promoters for these purposes include those from genes expressed in response to RSP virus infiltration.

The isolated DNA molecules of the present invention can be utilized to impart RSP virus resistance for a wide variety of grapevine plants. The DNA molecules are particularly well suited to imparting resistance to *Vitis* scion or rootstock cultivars. Scion cultivars which can be protected include those commonly referred to as Table or Raisin Grapes, such as Alden, Almeria, Anab-E-Shahi, Autumn Black, Beauty Seedless, Black Corinth, Black Damascus, Black Malvoisie, Black Prince, Blackrose, Bronx Seedless, Burgrave, Calmeria, Campbell Early, Canner, Cardinal, Catawba, Christmas, Concord, Dattier, Delight, Diamond, Dizmar, Duchess, Early Muscat, Emerald Seedless, Emperor, Exotic, Ferdinand de Lesseps, Fiesta, Flame seedless, Flame Tokay, Gasconade, Gold, Himrod, Hunisa, Hussiene, Isabella, Italia, July Muscat, Khandahar, Katta, Kourgane, Kishmishi, Loose Perlette, Malaga, Monukka, Muscat of Alexandria, Muscat Flame, Muscat Hamburg, New York Muscat, Niabell, Niagara, Olivette blanche, Ontario, Pierce, Queen, Red Malaga, Ribier, Rish Baba, Romulus, Ruby Seedless, Schuyler, Seneca, Suavis (IP 365), Thompson seedless, and Thomuscat. They also include those used in wine production, such as Aleatico, Alicante Bouschet, Aligote, Alvarelhao, Aramon, Baco blanc (22A), Burger, Cabernet franc, Cabernet, Sauvignon, Calzin, Carignane, Charbono, Chardonnay, Chasselas dore, Chenin blanc, Clairette blanche, Early Burgundy, Emerald Riesling, Feher Szagos, Fernao Pires, Flora, French Colombard, Fresia, Furmint, Gamay, Gewurztraminer, Grand noir, Gray Riesling, Green Hungarian, Green Veltliner, Grenache, Grillo, Helena, Inzolia, Lagrein, Lambrusco de Salamino, Malbec, Malvasia bianca, Mataro, Melon, Merlot, Meunier, Mission, Montua de Pilas, Muscadelle du Bordelais, Muscat blanc, Muscat Ottonel, Muscat Saint-Vallier, Nebbiolo, Nebbiolo fino, Nebbiolo Lampia, Orange Muscat, Palomino, Pedro Ximenes, Petit Bouschet, Petite Sirah, Peverella, Pinot noir, Pinot Saint-

George, Primitivo di Gioa, Red Veltliner, Refosco, Rkatsiteli, Royalty, Rubired, Ruby Cabernet, Saint-Emilion, Saint Macaire, Salvador, Sangiovese, Sauvignon blanc, Sauvignon gris, Sauvignon vert, Scarlet, Seibel 5279, Seibel 9110, Seibel 13053, Semillon, Servant, Shiraz, Souzao, Sultana Crimson, Sylvaner, Tannat, Teroldico, Tinta Madeira, Tinto cao, Touriga, Traminer, Trebbiano Toscano, Trousseau, Valdepenas, Viognier, Walschriesling, White Riesling, and Zinfandel. Rootstock cultivars which can be protected include Couderc 1202, Couderc 1613, Couderc 1616, Couderc 3309, Dog Ridge, Foex 33 EM, Freedom, Ganzin 1 (A x R #1), Harmony, Kober 5BB, LN33, Millardet & de Grasset 41B, Millardet & de Grasset 420A, Millardet & de Grasset 101-14, Oppenheim 4 (SO4), Paulsen 775, Paulsen 1045, Paulsen 1103, Richter 99, Richter 110, Riparia Gloire, Ruggeri 225, Saint-George, Salt Creek, Teleki 5A, Vitis rupestris Constantia, *Vitis california,* and *Vitis girdiana.*

Plant tissue suitable for transformation include leaf tissue, root tissue, meristems, zygotic and somatic embryos, and anthers. It is particularly preferred to utilize embryos obtained from anther cultures.

The expression system of the present invention can be used to transform virtually any plant tissue under suitable conditions. Tissue cells transformed in accordance with the present invention can be grown *in vitro* in a suitable medium to impart RSPaV resistance. Transformed cells can be regenerated into whole plants such that the protein or polypeptide imparts resistance to RSPaV in the intact transgenic plants. In either case, the plant cells transformed with the recombinant DNA expression system of the present invention are grown and caused to express that DNA molecule to produce one of the above-described RSPaV proteins or polypeptides and, thus, to impart RSPaV resistance.

In producing transgenic plants, the DNA construct in a vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference.

One technique of transforming plants with the DNA molecules in accordance with the present invention is by contacting the tissue of such plants

with an inoculum of a bacteria transformed with a vector comprising a gene in accordance with the present invention which imparts RSPaV resistance. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Bacteria from the genus *Agrobacterium* can be utilized to transform plant cells. Suitable species of such bacterium include *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*. *Agrobacterium tumefaciens* (e.g., strains C58, LBA4404, or EHA105) is particularly useful due to its well-known ability to transform plants.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by Agrobacterium and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium. Efficient regeneration will depend on the medium, on the genotype, and on the history

of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure so that the DNA construct is present in the resulting plants. Alternatively, transgenic seeds are recovered from the transgenic plants. These seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants.

Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., and in Emerschad et al., "Somatic Embryogenesis and Plant Development from Immature Zygotic Embryos of Seedless Grapes (*Vitis vinifera*)," Plant Cell Reports, 14:6-12 (1995) ("Emerschad (1995)"), which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Once a grape plant tissue is transformed in accordance with the present invention, it is regenerated to form a transgenic grape plant. Generally, regeneration is accomplished by culturing transformed tissue on medium containing the appropriate growth regulators and nutrients to allow for the initiation of shoot meristems. Appropriate antibiotics are added to the regeneration medium to inhibit the growth of *Agrobacterium* and to select for the

development of transformed cells. Following shoot initiation, shoots are allowed to develop tissue culture and are screened for marker gene activity.

The DNA molecules of the present invention can be made capable of transcription to a messenger RNA that does not translate to the protein. This is known as RNA-mediated resistance. When a *Vitis* scion or rootstock cultivar is transformed with such a DNA molecule, the DNA molecule can be transcribed under conditions effective to maintain the messenger RNA in the plant cell at low level density readings. Density readings of between 15 and 50 using a Hewlet ScanJet and Image Analysis Program are preferred.

A portion of one or more DNA molecules of the present invention as well as other DNA molecules can be used in a transgenic grape plant in accordance with U.S. Patent Application Serial No. 09/025,635, which is hereby incorporated herein by reference.

The RSPaV protein or polypeptide can also be used to raise antibodies or binding portions thereof or probes. The antibodies can be monoclonal or polyclonal.

Monoclonal antibody production may be effected by techniques which are well-known in the art. Basically, the process involves first obtaining immune cells (lymphocytes) from the spleen of a mammal (e.g., mouse) which has been previously immunized with the antigen of interest either *in vivo* or *in vitro*. The antibody-secreting lymphocytes are then fused with (mouse) myeloma cells or transformed cells, which are capable of replicating indefinitely in cell culture, thereby producing an immortal, immunoglobulin-secreting cell line. The resulting fused cells, or hybridomas, are cultured, and the resulting colonies screened for the production of the desired monoclonal antibodies. Colonies producing such antibodies are cloned, and grown either *in vivo* or *in vitro* to produce large quantities of antibody. A description of the theoretical basis and practical methodology of fusing such cells is set forth in Kohler and Milstein, Nature, 256:495 (1975), which is hereby incorporated by reference.

Mammalian lymphocytes are immunized by *in vivo* immunization of the animal (e.g., a mouse) with the protein or polypeptide of the present invention. Such immunizations are repeated as necessary at intervals of up to

several weeks to obtain a sufficient titer of antibodies. Following the last antigen boost, the animals are sacrificed and spleen cells removed.

Fusion with mammalian myeloma cells or other fusion partners capable of replicating indefinitely in cell culture is effected by standard and well-known techniques, for example, by using polyethylene glycol ("PEG") or other fusing agents. (See Milstein and Kohler, Eur. J. Immunol., 6:511 (1976), which is hereby incorporated by reference.) This immortal cell line, which is preferably murine, but may also be derived from cells of other mammalian species, including but not limited to rats and humans, is selected to be deficient in enzymes necessary for the utilization of certain nutrients, to be capable of rapid growth, and to have good fusion capability. Many such cell lines are known to those skilled in the art, and others are regularly described.

Procedures for raising polyclonal antibodies are also well known. Typically, such antibodies can be raised by administering the protein or polypeptide of the present invention subcutaneously to New Zealand white rabbits which have first been bled to obtain pre-immune serum. The antigens can be injected at a total volume of 100 μl per site at six different sites. Each injected material will contain synthetic surfactant adjuvant pluronic polyols, or pulverized acrylamide gel containing the protein or polypeptide after SDS-polyacrylamide gel electrophoresis. The rabbits are then bled two weeks after the first injection and periodically boosted with the same antigen three times every six weeks. A sample of serum is then collected 10 days after each boost. Polyclonal antibodies are then recovered from the serum by affinity chromatography using the corresponding antigen to capture the antibody. Ultimately, the rabbits are euthanized with pentobarbital 150 mg/Kg IV. This and other procedures for raising polyclonal antibodies are disclosed in Harlow et. al., editors, Antibodies: A Laboratory Manual (1988), which is hereby incorporated by reference.

In addition to utilizing whole antibodies, binding portions of such antibodies can be used. Such binding portions include Fab fragments, $F(ab')_2$ fragments, and Fv fragments. These antibody fragments can be made by conventional procedures, such as proteolytic fragmentation procedures, as

described in Goding, Monoclonal Antibodies: Principles and Practice, New York: Academic Press, pp. 98-118 (1983), which is hereby incorporated by reference.

The present invention also relates to probes found either in nature or prepared synthetically by recombinant DNA procedures or other biological procedures. Suitable probes are molecules that bind to RSP viral antigens identified by the polyclonal antibodies of the present invention or bind to the nucleic acid of RSPaV. Such probes can be, for example, proteins, peptides, lectins, or nucleic acids.

The antibodies or binding portions thereof or probes can be administered to RSPaV infected scion cultivars or rootstock cultivars. Alternatively, at least the binding portions of these antibodies can be sequenced, and the encoding DNA synthesized. The encoding DNA molecule can be used to transform plants together with a promoter which causes expression of the encoded antibody when the plant is infected by an RSPaV. In either case, the antibody or binding portion thereof or probe will bind to the virus and help prevent the usual stem pitting response.

Antibodies raised against the proteins or polypeptides of the present invention or binding portions of these antibodies can be utilized in a method for detection of RSPaV in a sample of tissue, such as tissue from a grape scion or rootstock. Antibodies or binding portions thereof suitable for use in the detection method include those raised against a replicase, proteins or polypeptides of the triple gene block, or a coat protein or polypeptide in accordance with the present invention. Any reaction of the sample with the antibody is detected using an assay system which indicates the presence of RSPaV in the sample. A variety of assay systems can be employed, such as enzyme-linked immunosorbent assays, radioimmunoassays, gel diffusion precipitin reaction assays, immunodiffusion assays, agglutination assays, fluorescent immunoassays, protein A immunoassays, or immunoelectrophoresis assays.

Alternatively, the RSPaV can be detected in such a sample using the DNA molecules of the present, RNA molecules of the present invention, or DNA or RNA fragments thereof, as probes in nucleic acid hybridization assays for detecting the presence of complementary virus DNA or RNA in the various tissue samples described above. The nucleotide sequence is provided as a probe in a nucleic acid

hybridization assay or a gene amplification detection procedure (e.g., using a polymerase chain reaction procedure). The nucleic acid probes of the present invention may be used in any nucleic acid hybridization assay system known in the art, including, but not limited to, Southern blots (Southern, E.M., "Detection of Specific Sequences Among DNA Fragments Separated by Gel Electrophoresis," J. Mol. Biol., 98:503-17 (1975), which is hereby incorporated by reference), Northern blots (Thomas, P.S., "Hybridization of Denatured RNA and Small DNA Fragments Transferred to Nitrocellulose," Proc. Nat'l Acad. Sci. USA, 77:5201-05 (1980), which is hereby incorporated by reference), and Colony blots (Grunstein, M., et al., "Colony Hybridization: A Method for the Isolation of Cloned cDNAs that Contain a Specific Gene," Proc. Nat'l Acad. Sci. USA, 72:3961-65 (1975), which is hereby incorporated by reference). Alternatively, the isolated DNA molecules of the present invention or RNA transcripts thereof can be used in a gene amplification detection procedure (e.g., a polymerase chain reaction). Erlich, H.A., et. al., "Recent Advances in the Polymerase Chain Reaction," Science 252:1643-51 (1991), which is hereby incorporated by reference. Any reaction with the probe is detected so that the presence of RSP virus in the sample is indicated. Such detection is facilitated by providing the DNA molecule of the present invention with a label. Suitable labels include a radioactive compound, a fluorescent compound, a chemiluminescent compound, an enzymatic compound, or other equivalent nucleic acid labels.

Depending upon the desired scope of detection, it is possible to utilize probes having nucleotide sequences that correspond with conserved or variable regions of the ORF or UTR. For example, to distinguish RSPaV from other related viruses (as described herein), it is desirable to use probes which contain nucleotide sequences that correspond to sequences more highly conserved among all RSPaV strains. Also, to distinguish between different RSPaV strains (e.g., RSPaV-1, RSP47-4, RSP158), it is desirable to utilize probes containing nucleotide sequences that correspond to sequences less highly conserved among the RSP virus strains.

Nucleic acid (DNA or RNA) probes of the present invention will hybridize to complementary RSPaV-1 nucleic acid under stringent conditions. Less stringent conditions may also be selected. Generally, stringent conditions are selected to be about 50°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic

strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. The T_m is dependent upon the solution conditions and the base composition of the probe, and may be calculated using the following equation:

$$
\begin{aligned}
T_m = 79.8°C \quad & + \quad (18.5 \times \mathrm{Log}[Na+]) \\
& + \quad (58.4°C \times \%[G+C]) \\
& - \quad (820 / \#bp \text{ in duplex}) \\
& - \quad (0.5 \times \% \text{ formamide})
\end{aligned}
$$

Nonspecific binding may also be controlled using any one of a number of known techniques such as, for example, blocking the membrane with protein-containing solutions, addition of heterologous RNA, DNA, and SDS to the hybridization buffer, and treatment with RNase. Generally, suitable stringent conditions for nucleic acid hybridization assays or gene amplification detection procedures are as set forth above. More or less stringent conditions may also be selected.

The development of a rapid detection method for RSP is a major breakthrough, because the only detection method now available is through inoculation of St. George grape indicators, which takes two to three years to develop symptoms. A serological or nucleic acid based detection tests developed for RSP will take only 1 to 2 days and it is less expensive. The woody indicator test on St. George costs $250 per sample, while a serological or nucleic acid based test would cost $30-50 per sample. Moreover, the rapid tests will speed up the introduction of grape imports into the US from the current three years to about six months. These applications will be valuable wherever grapes are grown. Since RSP is part of the rugose wood complex, development of rapid detection methods will be invaluable in determining the significance of RSP in the rugose wood complex. This will allow an investigator to determine whether RSP alone can cause the rugose wood complex or if other components are needed. In addition, these rapid detection methods are very useful to evaluate the resistance of transgenic plants to Rupestris stem pitting associated virus.

EXAMPLES

The following examples are provided to illustrate embodiments of the present invention but are by no means intended to limit its scope.

Example 1 - **Grapevine Materials for dsRNA Analysis**

Samples from 15 accessions that induced pitting on graft-inoculated St. George were collected from the National Grapevine Germplasm Repository of the USDA Plant Genetic Resources Unit (PGRU) at Geneva and used for dsRNA analysis. Positive controls used included Thompson Seedless (RSP105) (Golino, "The Davis Grapevine Virus Collection," Am. J. Enology Viticulture, 43:200-05 (1992), which is hereby incorporated by reference) from the FPMS, University of California (Davis) and Pinot Noir (SVP1186-09A2), which was kindly provided by Dr. R. Johnson of Center for Plant Health, Agriculture Canada, Sidney, British Columbia. Negative controls as judged by indexing on St. George included Freedom from the PGRU at Geneva, New York, and Verduzzo 233A. The latter was kindly provided by Dr. P. Silvano of the Sezione di Fitovirologia, ERSA Servizio Chimico-Agrario e della Certificazione, Pozzuolo del Friuh (UD), Italy.

Example 2 - **Grapevine Materials for RT-PCR**

Dormant cuttings of 138 grapevine selections were collected from USA, Canada, Italy, and Portugal over three years. Samples included *Vitis* vinifera cultivars, hybrids, V. riparia, and rootstocks. 117 grapevine selections were indexed on St. George for RSP and other RW diseases. Pinot noir (1186-9A2) from Agriculture Canada, Center for Plant Health (Sidney, Canada) and Thompson seedless (RSP105) from University of California (Davis) were included as positive controls. Sauvignon blanc, generated from shoot tip tissue culture and tested free of viruses and viroids was provided by Dr. J. Semancik (University of California at Riverside) and used as a healthy control. In addition, six seedlings of five *Vitis* species were also included as negative controls.

Example 3 - **dsRNA Isolation and Analysis**

Methods for isolating dsRNA were described by Hu et al., "Characterization of Closterovirus-like Particles Associated with Grapevine Leafroll Disease," J. Phytopathology, 128:1-14 (1990), which is hereby incorporated by

reference, except that 1 X STE with 15% ethanol (instead of 16.5%) was used to wash CF-11 cellulose columns prior to elution of dsRNAs. The dsRNAs were isolated from leaves, petioles, and the phloem tissue of dormant canes, electrophoresed on 1% agarose or low melting temperature agarose gels, and analyzed by staining with ethidium bromide (EtBr). *Hind* EII digested lambda DNA was used as markers to estimate the sizes of the dsRNA molecules.

Example 4 - cDNA Synthesis and Cloning

The extremely low yield of dsRNA and the limited quantity of RSP-infected grape materials precluded the use of a single RSP-infected grapevine accession as the source of dsRNA for cloning purpose. Therefore, dsRNA preparations from Colobel 257, Ravat 34, Couderc 28-112, and Seyval were pooled and used as templates for cDNA synthesis. In order to get pure templates for cloning, dsRNA bands were excised from low melting temperature agarose gels after electrophoresis and recovered by extraction with phenol and chloroform (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989), which is hereby incorporated by reference). The same recovery procedure was repeated once more. The purified dsRNA was denatured with 20 mM methyl mercuric hydroxide and cDNAs were synthesized using slightly modified methods of Jelkmann et al., "Cloning of Four Viruses from Small Quantities of Double-Stranded RNA," Phytopathology, 79:1250-53 (1989), which is incorporated herein be reference. The cDNA fragments were first blunt-ended with T4 DNA polymerase at 12°C. T4 DNA ligase was used to add *EcoR* I adapters to both ends of the cDNAs. Subsequently, the cDNA molecules with cohesive ends were ligated to EcoR I-prepared arms of lambda ZAP II. Finally, the resulting recombinant phages were packed into Gigapack II packaging extract following manufacturer's instructions (Stratagene, La Jolla, CA).

Example 5 - Identification of cDNA Clones Specific to the dsRNA

Plaque hybridization was used to screen cDNA clones by transferring recombinant cDNA plaques to nylon membranes and hybridizing to ^{32}P-labeled first-

strand cDNA probes generated from the dsRNA according to manufacturer's recommendations (Du Pont, 1987). Clones with strong hybridization signals were converted into pBluescript SK through *in vivo* excision (Stratagene, 1991). After digestion of the resulting plasmids with *EcoR* I, 20 clones were selected and further analyzed in Southern hybridization with radio labeled first strand cDNA probes synthesized from the dsRNA. The specificity of two selected clones to the dsRNA was confirmed by Northern analysis using ^{32}P labeled inserts of the two clones.

Example 6 - Bridging Gaps Between Clones

To bridge the gap between clones RSP3 and RSP94, a pair of specific primers were used in RT-PCR to generate cDNA fragments from the dsRNA. RSP3-RSP94 primer 1 (sense, nt 3629-3648) has a nucleotide sequence corresponding to SEQ. ID. No. 41 as follows:

GCTTCAGCAC TTGGAAGGCG 20

RSP3-RSP94 primer 2 (antisense, nt 4350-4366) has a nucleotide sequence corresponding to SEQ. ID. No. 42 as follows:

CACACAGTGG CCAGCCT 17

After gel electrophoresis, PCR amplified cDNA bands were excised from gels and recovered with the phenol/chloroform method (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989), which is hereby incorporated by reference).

The same strategy was employed to bridge the gap between clones RSP94 and RSP95. RSP94-RSP95 primer 1 (sense, nt 5272-5291) has a nucleotide sequence corresponding to SEQ. ID. No. 43 as follows:

GGAGGTGCGT TGTGGTTATG 20

RSP94-RSP95 primer 2 (antisense, nt 6791-6808) has a nucleotide sequence corresponding to SEQ. ID. No. 44 as follows:

CCCTGGCACT GCACACCC 17

Example 7 - Obtaining Nucleotide Sequences on Both Termini of RSPaV-1 Genome

To obtain the terminal 3' end sequences, a primer (sense, nt 8193-8210) having a nucleotide sequence corresponding to SEQ. ID. No. 45 as follows:

```
GGAGGTGACC ACATTACG                                          18
```

and a (dT)18 primer were used in RT-PCR to amplify cDNA from the dsRNA. Resulting PCR products were cloned into TA vector pCRII (Invitrogen) and sequenced. This approach was based on the assumption that the RSP associated dsRNA contained a poly (A) tail. For the terminal 5' end, the dsRNA was first tagged with poly (A) using yeast Poly (A) polymerase (USB) (Pappu et al., "Nucleotide Sequence and Organization of Eight 3' Open Reading Frames of the Citrus tristeza Closterovirus Genome," Virology 199:35-46 (1994), which is hereby incorporated by reference) and then used as templates to generate cDNA fragments by RT-PCR using (dT)18 primer and primer (antisense, nt 429-449) having a nucleotide sequence corresponding to SEQ. ID. NO. 46 as follows:

```
CATCACGACT TGTCACAAAC C                                      21
```

Example 8 - Nucleotide Sequencing

CsCl or alkaline/PEG (polyethylene glycol) purified plasmids (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989), which is hereby incorporated by reference; Applied Biosystems, Inc.) and RT-PCR amplified cDNA fragments were sequenced for completion on both strands. Nucleotide sequencing was done manually with Sequenase version 2.0 kit (USB) or automatically on ABI 373 automated sequencer with Taq DyeDeoxy™ terminator cycle sequencing kit (Applied Biosystems, Inc.). Vector primers (T3, T7, M13 Forward, and M13 Reverse) were used in initial sequencing and sequences were completed by primer walking strategy.

Example 9 - **Reverse Transcription-Polymerase Chain Reaction (RT-PCR)**

Two pairs of primers were designed for RT-PCR: (1) RSP95F1 and RSP95R1; and (2) RSP149F1 and RSP149R1. Primer RSP95F1, an antisense strand primer, has a nucleotide sequence corresponding to SEQ. ID. NO. 47 as follows:

```
TGGGCCTCCA CTTCTTC                                                17
```

Primer RSP95R1, a sense strand primer, has a nucleotide sequence corresponding to SEQ. ID. No. 48 as follows:

```
GGGGTTGCCT GAAGAT                                                 16
```

Primer RSP149F1, an antisense strand primer, has a nucleotide sequence corresponding to SEQ. ID. No. 49 as follows:

```
ACACCTGCTG TGAAAGC                                                17
```

Primer RSP149R1, a sense strand primer, has a nucleotide sequence corresponding to SEQ. ID. No. 50 as follows:

```
GGCCAAGGTT CAGTTTG                                                17
```

RSP95F1/R1 were used in RT-PCR to test samples collected in 1994. RSP149R1/F1, alone or together with RSP95F1/R1, were used to test samples collected in 1995 and 1996. To avoid bias in the judgment of RT-PCR results, blind tests were conducted for samples from Canada in 1995 and 1996. The indexing results of these samples were kept untold until the RT-PCR tests were complete.

dsRNAs were denatured with methylmercuric hydroxide (CH4HgOH) and reverse transcribed into cDNAs with Moloney murine leukemia virus (MMLV) or Avian Myeloblastosis Virus (AMV) reverse transcriptases (Promega) at 42 ^{o}C for 1 to 3 h. Five of 20 μl of the RT reactions were added to PCR mix and amplified in thermal cycler (HYBAID OmniGene, National Labnet Company) with *Taq* DNA polymerase (buffer B, Promega) using the following parameters: initial denaturation at 94 ^{o}C for 5 min, 40 cycles of amplification at 94 ^{o}C for 45 s, 52 ^{o}C for 1 min, and 72 ^{o}C for 1 min, and a final extension at 72 ^{o}C for 10 min. PCR products were analyzed by electrophoresis on 1% agarose gels containing ethidium bromide. Hae III digested Phix 174 fragments were used as molecular weight markers.

Example 10 - Southern Blot

DNA fragments amplified by PCR from cDNA clone RSP149 with primers RSP149F1/R1 were labeled with 32P by random priming and used as probes. Products of RT-PCR of randomly selected grapevines including 26 positives and 6 negatives by RT-PCR were electrophoresed on an 0.8% agarose gel, transferred to nylon membranes, and hybridized to the probes following manufacturer's instructions (Du Pont).

Example 11 - Computer Assisted Analysis of Sequences and Genome Structure of RSPaV-1

Sequences were assembled with SeqMan program and potential open reading frames were generated with MapDraw program (DNASTAR, Madison, WI). BLAST program of the NCBI (the National Center for Biotechnology Information) was used to search for homologies in DNA and protein databases. Clustal analysis (with identity weight table) of MegAlign (DNASTAR) was employed to reveal sequence similarities between the putative proteins of RSPaV-1 and the analogous proteins of ASPV (Jelkmann, "Nucleotide Sequences of Apple Stem Pitting Virus and of the Coat Protein of a Similar Virus from Pear Associated with Vein Yellows Disease and Their Relationship with Potex- and Carlaviruses," J. General Virology, 75:1535-42 (1994), which is hereby incorporated by reference) and PVM (Zavriev et al., "Complete Nucleotide Sequence of Genomic RNA of the Potato M-Virus," Molecular Biology (Mosk.) 25:761-69 (1991), which is hereby incorporated by reference). In addition, nucleotide sequences of the untranslated regions (UTR) of these three viruses were also compared using MagAlign, as shown in Figures 6A and 6B.

Example 12 - Consistent Association of a High Molecular Weight dsRNA with RSP

The 15 grapevine accessions used in this study were previously indexed on St. George where 12 accessions induced typical RSP symptoms (i.e., a narrow strip of small pits below the inoculum bud). Figure lA illustrates these

typical RSP symptoms. A good correlation was found between the presence of the specific dsRNA and the indexing results on St. George. As shown in Figure 2A and recorded in Table 1 below, twelve grapevine accessions with typical RSP symptoms revealed a dsRNA of ca. 8.7 kb with gel electrophoresis. In addition, a smaller dsRNA of about 6.6 kb was observed in Colobel 257 and Seyval. In contrast, although Aminia and Canandaigua elicited deep pits and grooves around the woody cylinder of St. George, they did not reveal visible dsRNA of expected size in repeated experiments. Freedom, which indexed negative for RSP on St. George, did not reveal visible dsRNA. Although two dsRNA bands were observed in Verduzzo 233A (which was indexed free of RSP on St. George), they were not specific to RSP based on the fact that they were larger or smaller than the 8.7 kb dsRNA associated with RSP (Figure 2A) and that they did not hybridize to the RSP-specific probe in Northern analysis (Figure 2B). In addition, the two dsRNA species isolated from Verduzzo 233A were not observed in other healthy grapevines such as Cabernet Franc and LN 33.

Table 1

Accessions and Parentage	St. George Indicator	dsRNA	Northern
Aminia (Carter X Black Hamburg)	+	–	–
Bertille Seyve 3408 (BS 872 X Seibel 5410)	+	+	+
Bertille Seyve 5563 (Seibel 6905 X BS 3445)	+	+	+
Canandaigua (*V. labrusca* X *V. vinifera*)	+	–	–
Colobel 257 (Seibel 6150 X Seibel 5455)	+	+	+
Couderc 28-112 (Emily X *V. rupestris*)	+	+	+
Freedom (Couderc 1613 X Dog Ridge)	–	–	–
Grande Glabre (*V. riparia*)	+	+	+
Ill 344-1 (BS 2667 X Seibel 6905)	+	+†	–†
Joffre (*V. vinifera* X *V. riparia* X *V. rupestris*)	+	+	+
Ravat 34 (Berlandieri X Chardonnay)	+	+	+
Seyval (Seibel 4995 X Seibel 4986)	+	+	+
Seyve Villard 14-287 (*V. labrusca* X *V. rupestris* X *V. aestiv* X *V. cinerea* X *V. vinifera*)	+	+	+
Seyve Villard 3160 (Seibel 5163 X Seibel 2049)	+	+	+
Verdelet (Seibel 5455 X Seibel 4938)	+	+	+
Controls			
Pinot Noir (*V. vinifera*)	+	+	+

Table 1

Accessions and Parentage	St. George Indicator	dsRNA	Northern
Thompson seedless (*V. vinifera*)	+	NT	+
Verduzzo 233A	–	–‡	–

Symbols:
* Probe used was insert from cDNA clone RSP149.
† A faint dsRNA band could be observed on the gel after electrophoresis but no hybridization signal could be seen in Northern analysis.
‡ Although two dsRNA bands were observed in Verduzzo 233A, they were not specific to RSP, because they were either larger or smaller than the RSP-associated 8.7 kbp dsRNA and they did not hybridize to the probe in Northern analysis.

The yield of dsRNA was low and varied significantly among different accessions. When a comparable amount of phloem tissue (14 g for Bertille Seyve 5563 and Couderc 28-112; 18.5 g for the others) was used to isolate dsRNA, Colobel 257, Seyval, Ravat 34, Grande Glabre, and Seyve Villard 14-287 displayed strong dsRNA bands, while Bertille Seyve 5563, Couderc 28-112, Joffre, and Verdelet showed weak bands after staining with EtBr, as shown in Figure 2A. Bertille Seyve 3408 and Seyve Villard 3160 were analyzed in separate experiments and dsRNA bands of the same size were observed.

Example 13 - **Selection and Specificity of cDNA Clones**

A total of 182 clones were selected after plaque hybridization. Eighty clones with strong hybridization signals were subcloned into pBluescript SK through *in vivo* excision. Resulting plasmids were shown to have inserts ranging from 0.3 to 3.0 kb. A total of 20 clones with inserts of ca. 0.8 kb or larger were selected. Southern analysis of these 20 clones to radio labeled first strand cDNA probes derived from the dsRNA resulted in 15 clones with strong hybridization signals. Several of these clones were used to determine the genome sequence of the dsRNA: RSP3, RSP28, RSP94, RSP140, RSP95, and TA5. Another clone (RSP149), which was 97% similar in nucleotide sequence to RSP95, was used as one of the two probes in Northern hybridization.

Northern hybridization was employed to confirm the specific relationship of clones RSP95 and RSP149 to the isolated dsRNA. These two clones gave the strongest reaction in Southern analysis described above. Initial experiments showed that RSP95 insert hybridized with the dsRNA isolated from three accessions

(Colobel 257, Seyval, and Ravat 34), from which the template dsRNAs used in cDNA synthesis were isolated. As shown in Figure 2B and indicated in Table 1, use of RSP149 insert as the probe showed that this clone hybridized with the dsRNA of ca. 8.7 kb isolated from RSP infected grapevines. Furthermore, the intensity of hybridization signals corresponded to that of the dsRNA bands observed on agarose gels stained with EtBr. Colobel 257, Seyval, Ravat 34, Grande Glabre, and Serve Villard 14-287 reacted strongly; Bertille Seyve 5563, Couderc 28-112, Joffre, and Verdelet had weak hybridization signals. The result for Ill 344-1 was not conclusive. Aminia and Canandaigua did not show visible dsRNAs or hybridization in Northern analysis. Bertille Seyve 3408, which was tested in a separate experiment, did show a ca. 8.7 kb dsRNA which hybridized to the probe from RSP149. Freedom and Verduzzo 233A, which had indexed negative for RSP on St. George, were also negative in Northern blot.

Example 14 - Nucleotide Sequence and Genome Structure of RSPaV-1

Six cDNA clones and three RT-PCR amplified cDNA fragments (identified as RSPA, RSPB, and RSPC) were sequenced on both strands and used to obtain the complete nucleotide sequence of a viral agent, which is shown in Figure 3A. The genome of RSPaV-1 consisted of 8726 nts excluding a poly (A) tail on the 3' end. The sequence of RSPA indicated that the 5' first base of the RSPaV-1 genome appeared to be a cytosine (C). Clone TA5, which represented the 3' end of the RSPaV-1 genome, contained a stretch of adenines (A) preceded by a cytosine.

MapDraw analysis, shown at Figure 3B, indicated that the genome of RSPaV-1 had five potential ORFs on its positive strand, while no ORFs were observed on the negative strand (data not shown). ORF1 (nt 62 to 6547 of SEQ. ID. No. 1) has a nucleotide sequence corresponding to SEQ. ID. NO. 2. ORF1 believed to encode a protein or polypeptide having a molecular weight of about 244 kDa and an amino acid sequence corresponding to SEQ. ID. No. 3. According to Lutcke et al., "Selection of AUG Initiation Codons Differs in Plants and Animals," Eur. Mol. Biol. J., 6:43-48 (1987), which is hereby incorporated by reference, the start codon of ORF1 was in a favorable context: GCAAUGGC, where the "GC" after the start codon is important for initiating translation in a plant system. ORF2 (nt 6578 to 7243 of

SEQ. ID. No. 1) has a nucleotide sequence corresponding to SEQ. ID. No. 4. ORF2 is believed to encode a protein or polypeptide having a molecular weight of about 24.4 kDa and an amino acid sequence corresponding to SEQ. ID. NO. 5. The first two ORFs were separated by an intergenic region of 30 nts. ORF3 (nt 7245 to 7598 of SEQ. ID. NO. 1) has a nucleotide sequence corresponding to SEQ. ID. No. 6. ORF3 is believed to encode a protein or polypeptide having a molecular weight of about 12.8 kDa and an amino acid sequence corresponding to SEQ. ID. NO. 7. ORF4 (nt 7519 to 7761 of SEQ. ID. NO. 1), which overlapped with ORF3 by 80 nts, has a nucleotide sequence corresponding to SEQ. ID. No. 8. ORF3 is believed to encode a protein or polypeptide having a molecular weight of about 8.4 kDa and an amino acid sequence corresponding to SEQ. ID. No. 9. Nine nucleotides downstream of ORF4 was the start of ORF5 (nt 7771 to 8550 of SEQ. ID. No. 1), which has a nucleotide sequence corresponding to SEQ. ID. No. 10. ORF5 is believed to encode a protein or polypeptide having a molecular weight of about 28 kDa and an amino acid sequence corresponding to SEQ. ID. No. 11. Downstream of ORF5 was the 3' end LJTR of 176 nts. Although computer assisted analysis indicated that two shorter ORFs may exist as alternatives to ORF1 and ORF5, neither of them were in good contexts for translation initiation.

<u>Example 15</u> - Comparison of the RSPaV-1 Genome with ASPV and PVM Carlavirus Genomes

The arrangement of the ORFs and the amino acid sequences of RSPaV-1 showed similarities to those of PVX (Skryabin et al., "The Nucleotide Sequence of Potato Virus X RNA," <u>Nucleic Acids Res.</u> 16: 10929-30 (1988), which is hereby incorporated by reference), PVM (Zavriev et al., "Complete Nucleotide Sequence of Genomic RNA of the Potato M-Virus," <u>Molecular Biology (Mosk.)</u> 25:761-69 (1991), which is hereby incorporated by reference), and ASPV (Jelkmann, "Nucleotide Sequences of Apple Stem Pitting Virus and of the Coat Protein of a Similar Virus from Pear Associated with Vein Yellows Disease and Their Relationship with Potex- and Carlaviruses," <u>J. General Virology</u> 75:1535-42 (1994), which is hereby incorporated by reference), with the latter two being the most similar to RSPaV-1. A representation of the sequence comparison is shown in Figure 3B and the percent identities in amino acid sequences of the ORF of RSPaV-1 and the

corresponding ORF of ASPV, PVM, and PVX are shown in Table 2 below. These analyses suggest that the ORFs of RSPaV-1 are compared with those of PVM and ASPV.

Table 2

	Replicase			Triple Gene Block				Coat Protein
	Region I aa 1-372	ORF1 Region II aa 1354-2161	Total	ORF2	ORF3	ORF4	Total	ORF5 aa142-245
ASPV	49.2	57.5	39.6	38.0	39.3	27.1	31.3	49.5
PVM	47.2	53.2	37.6	34.8	31.2	19.0	21.2	33.3
PVX	18.9	20.4	15.7	23.5	31.3	22.9	27.4	42.9

When the total amino acid sequence of RSPaV-1 ORF1 was used for comparison, it showed 39.6% and 37.6% identities with the replicases of ASPV and PVM respectively (Table 2). These homologies were mainly found in regions I (aa 1 to 372) and II (aa 1354-2161), which are at the N and C terminal portions of the putative replicase, respectively, shown at Figures 4A and 4B. Within region I, the identities of RSPaV-1 with ASPV and PVM were 49.2% and 47.2%, respectively (Table 2). The methyltransferase domain, which is conserved in Sindbis-like superfamily of plant viruses (Rozanov et al., "Conservation of the Putative Methyltransferase Domain: A Hallmark of the "Sindbis-like" Supergroup of Positive-Strand RNA Viruses," J. General Virology 73:2129-34 (1992), which is hereby incorporated by reference), was found in this region (Figure 4A). Region II, on the other hand, showed even higher identities: 57.5% with ASPV and 53.2% with PVM (Table 2). A NTP binding motif "GXXXXGKS/T" (aa 1356 to 1363) ("X" stands for any amino acid residue), which is conserved in helicase proteins and helicase domains of eukaryotic positive strand RNA viruses (Gorbalenya et al., "A Novel Superfamily of Nucleotide Triphosphate-Binding Motif Containing Proteins which are Probably Involved in Duplex Unwinding in DNA and RNA Replication and Recombination," FEBS Letters, 235:16-24 (1988), which is hereby incorporated by reference), was found in the beginning of region II (Figure 4B). The amino acid sequences of this motif in ASPV and PVM were identical to that of RSPaV-1 except for one position. Furthermore, amino acid sequence surrounding the GDD motif, which is conserved in all RNA dependent RNA polymerases of positive strand RNA viruses (Koonin, "The

Phylogeny of RNA-Dependent RNA Polymerases of Positive-Strand RNA Viruses," J. Gen. Virology 72:2197-2206 (1991), which is hereby incorporated by reference), was located near the C terminus of the RSPaV-1 replicase protein and showed high identities to those of ASPV and PVM (Figure 4B). Other conserved residues of positive strand RNA viruses as described by Koonin, "The Phylogeny of RNA-Dependent RNA Polymerases of Positive-Strand RNA Viruses," J. Gen. Virology 72:2197-2206 (1991), which is hereby incorporated by reference, were also found in this region. Based on these information, it was concluded that ORF1 of RSPaV-1 codes for the putative replicase protein.

The triple gene block is a common feature of several groups of plant viruses including carlaviruses, potexviruses, and ASPV. Comparison of RSPaV-1 ORF2 with those of PVM and ASPV showed evenly distributed homologies in amino acid sequence: 38.0% identity to ASPV and 34.8% to PVM (Table 2). The N terminal region of the 24.4K protein (ORF2) contained the consensus sequence "GXGKS S/T" (aa 31 to 36) (Figure 5A), which is observed in its counterparts in carlaviruses (Zavriev et al., "Complete Nucleotide Sequence of Genomic RNA of the Potato M-Virus," Molecular Biology (Mosk.) 25:761-69 (1991), which is hereby incorporated by reference) and a number of ATP and GTP binding proteins (Zimmem, "Evolution of RNA Viruses," in RNA Genetics, Holland et al., eds., CRC Press, Boca Raton, Florida, USA (1987), which is hereby incorporated by reference). The 12.8K protein of RSPaV-1 encoded by ORF3 had 39.3% and 31.2% identities with its counterparts in ASPV and PVM respectively (Table 2). However, most of the matching occurred in a region from aa 29 to 62, among which 18 aa were fully conserved in all three viruses (Figure 5B). These 12-13K proteins may function in membrane binding (Morozov et al., "Nucleotide Sequence of the Open Reading Frames Adjacent to the Coat Protein in Potato Virus X Genome," FEBS Letters 213:438-42 (1987), which is hereby incorporated by reference). The 8.4K protein encoded by RSPaV-1 ORF4, in contrast, showed much lower identities: 27.1% with that of ASPV and 19.0% with that of PVM (Table 2). However, four residues "TGES" (aa 38 to 41) were conserved in all three viruses (Figure 5C). *In vitro* studies indicated that the analogous 7K protein of PVM may bind to single or double stranded nucleic acids (Gramstat et al., "The 12 kDa Protein of Potato Virus M Displays Properties of a Nucleic Acid-Binding Regulatory Protein," FEBS Letters, 276:34-38 (1990), which is hereby

incorporated by reference) and to plasma membrane (Morozov et al., "In vitro Membrane Binding of the Translation Products of the Carlavirus 7-kDa Protein Genes," Virology 183:782-85 (1991), which is hereby incorporated by reference).

A sequence similarity search in a DNA database revealed identities between the putative protein encoded for by RSPaV-1 ORF5 to the coat proteins (CPs) of several groups of plant viruses, indicating that RSPaV-1 ORF5 may code for the coat protein. MegAlign analysis revealed that RSPaV-1 ORF5 had 31.3% and 21.2% identities with the CPs of ASPV and PVM, respectively (Table 2). Most of the identities were found in the C terminal portion of the coat proteins (aa 142 to 245 for RSPaV-1), while the N terminal portions were quite variable in the numbers and sequences of amino acid residues. When the C terminal portion of RSPaV-1 CP was compared to the corresponding regions of ASPV and PVM, it showed 49.5% and 33.3% identities with ASPV and PVM, respectively (Table 2). In addition, the "RR/QX-XFDF" motif was found in the central region of RSPaV-1 CP (Figure 5D). This motif is conserved in the CPs of positive strand RNA viruses with filamentous morphology and were reported to be involved in salt bridge formation (Dolja et al., "Phylogeny of Capsid Proteins of Rod-Shaped and Filamentous RNA Plant Virus: Two Families with Distinct Patterns of Sequence and Probably Structure Conservation," Virology, 184:79-86 (1991), which is hereby incorporated by reference). Therefore, it is believed that ORF5 encodes a putative coat protein.

MegAlign analysis, shown in Figures 6A and 6B, revealed that the 3' UTR of RSPaV-1 is more similar to that of PVM than to that of ASPV. For example, in a 75 nts stretch, RSPaV-1 had 68% identity with PVM. Within this region, 21 consecutive nucleotides were identical between these two viruses. The significance of this conservation in nucleotide sequence remains to be explored. In contrast, the 5' UTR of RSPaV-1 did not reveal significant similarities with those of PVM and ASPV.

It has been have shown that an 8.7 kbp dsRNA is consistently associated with grapevines that indexed positively on St. George for RSP. Sequence analyses of the dsRNA provide evidence that a virus is involved in RSP, which has now been named RSPaV-1. The complete nucleotide sequence of RSPaV-1 was determined from overlapping cDNA clones and RT-PCR-amplified cDNA fragments generated from the dsRNA. The RSPaV-1 genome has five ORFs coding for the

putative replicase (ORF1), the triple gene block (ORF2-4), and the CP (ORF5). The existence of these ORFs and their potential to code for structural and non-structural viral proteins were further supported by the identification of conserved motifs which are the signatures of various viral proteins.

This work confirms and extends the findings of Walter and Cameron ("Double-stranded RNA Isolated from Grapevines Affected by *Rupestris* Stem Pitting Disease," Am. J. Enology and Viticulture 42:175-79 (1991), which is hereby incorporated by reference), and Azzam and Gonsalves ("Detection of dsRNA in Grapevines Showing Symptoms of Rupestris Stem Pitting Disease and the Variabilities Encountered," Plant Disease 75:960-64 (1991), which is hereby incorporated by reference), who observed a major dsRNA species of about 8.0-8.3 kbp in RSP-infected grapevines. In addition, such work also observed a smaller dsRNA of ca. 6.6 kbp. A dsRNA of similar size was also observed here, but it was consistently detected in only Colobel 257 and Seyval. The relationship, if any, of this smaller dsRNA to RSP remains to be determined. The small dsRNA of ca. 0.359 kbp, which Monette et al. ("Double-stranded RNA from *Rupestris* Stem Pitting-Affected Grapevines," Vitis 28:137-44 (1989), which is hereby incorporated by reference) isolated from RSP-infected grapevines growing in tissue culture, was not observed.

Electron microscopy evidence also suggests that RSP is caused by filamentous virus(es). Tzeng et al. ("Anatomical and Tissue Culture Studies of Rupestris Stem Pitting-Affected Grapevines," Botan. Bulletin of Acad. Sinica (Taipei) 34:73-82 (1993), which is hereby incorporated by reference) observed flexuous filamentous virus aggregates in the phloem parenchyma cells of young shoots of Sylvner grapevines that had indexed positively for RSP. Monette and Godkin ("Detection of Capillovirus-like Particles in a Grapevine Affected with Rugose Wood," Vitis 34:241-42 (1995), which is hereby incorporated by reference) observed a filamentous virus in Sauvignon blanc infected by RSP and LNSG. The relationship of these virus particles to RSP disease remains to be studied.

Evidence suggests that the cDNA library generated from the isolated dsRNA templates is not homogeneous for only RSPaV-1. During the process of

sequencing cDNA clones, several clones (e.g., RSP47-4 and RSP158) were identified with high, but not identical, sequence similarities to RSPaV-1.

RSPaV-1 has the most similarities to ASPV, which has not yet been grouped into a virus genus. Both viruses have the same genome organization and their ORFs code for putative proteins of similar sizes, except that the coat protein of ASPV is significantly larger (44 kDa) than that of RSPaV-1 (28 kDa). Comparisons of RSPaV-1 with PVM carlavirus show some similarities in genome organization except that RSPaV-1 lacks ORF6 which is located at the 3' end of PVM genome. Although the genome organization of RSPaV-1 is similar to PVX potexvirus, the latter has a much smaller putative replicase. RSPaV-1 has no relation to grape viruses whose genomes have been sequenced so far. The closest possibilities, GVA (Minafra et al., "Grapevine virus A: Nucleotide Sequence, Genome Organization, and Relationship in the *Trichovirus* Genus," Arch. Virology 142:417-23 (1997), which is hereby incorporated by reference) and GVB (Saldarelli et al., "The Nucleotide Sequence and Genomic Organization of Grapevine Virus B," J. General Virology 77:2645-52 (1996), which is hereby incorporated by reference), have different genome structures than RSPaV-1.

Example 16 - Specific and Universal Primers and the Detection of Different Strains of RSPaV by Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Among the 138 grapevine entries collected, 25 indexed negatively and 93 indexed positively for RSP on St. George, while the others were not indexed (see Tables 3-7 below). Symptoms induced by RSP on the woody cylinder of St. George after graft inoculation with chip-buds can be divided into two types. The first type is called "specific", that is, pits and/or grooves being restricted to the area on the woody cylinder below the inoculation sites. The other is called "nonspecific", that is, pits and/or grooves being present above, around, and below the inoculation sites.

Table 3

Cultivar/Accession	ID	Index St.G	RT-PCR	Source
Almeria K3 P 661	1483-13D1	-	-	C
Auxerrois CL 56	658-1A2	-	-a	C
Auxerrois CL 56	658-1A1-1A2	-	-	C
GM 32458	604-8A2-2A2	-	-	C
GM 7117-10	1347-16A1	-	-a	C

Table 3

Cultivar/Accession	ID	Index St.G	RT-PCR	Source
Italia	1186-5B1	-	-	C
Pslanka (H)	23-10A2-2A2	-	-	C
Ventura (V. 51061) (H)	1166-2A1	-	-	C
Verdelet (H)	1170-3C2-2S1	-	-	C
Verduzzo (V)	233A	-	-	I
Vivant (V. 63331) (H)	1166-3A1	-	-	C
Control				
Sauvignon Blanc (V)	AV-4 #2	-	-a	U

Symbols:
V., *Vitis vinefera*; R., *Vitis riparia*; H., hybrid; C., Canada; I., Italy; U., USA; P., Portugal;
a, tested by RSP149F1/R1 and 95F1/R1 and results agree to each other; b, tested by 95F1/R1 only

Table 4

Cultivar/Accession	ID	Index St.G	RT-PCR	Source
Aragonez (Temperanillo)	238	-	+	P
Albalonga	1058-4A2-2A1	-	+	C
Cabernet Franc (V)	147A	-	+	I
Chardonnay (V)	80A	-	+	I
Ehrenfelser PM 1 (V)	1169-1A1	-	+	C
Freedom (H)	PI 588370	-	+a	U
Harslevellu P 679	1483-2B1	-	+	C
Heroldrebe	1318-2A1	-	+	C
Malvasia Fina	340	-	+	P
Perle of Zala	1407-5A1	-	+	C
Refosco (V)	181A	-	+	I
San Giovese Brunello CL BBS 11	1497-2A1	-	+	C
Touriga Francesa	313	-	+	P

Symbols:
V., *Vitis vinefera*; R., *Vitis riparia*; H., hybrid; C., Canada; I., Italy; U., USA; P., Portugal;
a, tested by RSP149F1/R1 and 95F1/R1 and results agree to each other; b, tested by 95F1/R1 only

Table 5

Cultivar/Accession	ID	Index St.G	RT-PCR	Source
Albalonga	1058-4A2-1A2	+	+	C
Aminia (H)	PI 588306	+	+	U
Antao Vaz	CL 245	+	+	P
Aragonez (Temperanillo)	350	+	+	P
Auxerrois CL 56	658-1A1	+	+	C
Badacsony-10	1407-1A1	+	+	C
Bertille Seyve 3408 (H)	GVIT 348	+	+b	U
Bertille Seyve 5563 (H)	PI 181647	+	+a	U
Blauer Spatburgunder	Q1378-1	+	+b	C
Blauer Zwiegelt/5BB	1240-1A1	+	+a	C
Bonbino B 9	1586-17P3	+	+	C
Brant (H)	1078-1A1	+	+	C
Cabernet Franc (V)	151A	+	+	I
Cabernet Sauvignon (V)	124A	+	+	I
Cardinal	Q390-13	+	+b	C
Chardonnay (V)	Q661-4	+	+b	C

Table 5

Cultivar/Accession	ID	Index St.G	RT-PCR	Source
Chardonnay CL 116 (V)	1021-13A2	+	+a	C
Chardonnay (V)	128B	+	+b	I
Chardonnay (V)	72A	+	+b	I
Chardonnay (V)	73A	+	+b	I
Chardonnay (V)	83A	+	+	I
Chazan CL 538	1346-6A1	+	+a	C
Chenin Blanc CL 220	1555-6A1	+	+	C
Colobel 257 (Seibel 8357) (H)	PI 588062	+	+a	U
Couderc 28-112 (H)	PI 588248	+	+a	U
De Chaunac S9549 (H)	Q659-1	+	+b	C
Durella 3	1586-13P1	+	+	C
Esgana cao	276	+	+	P
Egri Csillagok-30	1407-3A1	+	+	C
Gamay Precoce	1500-2A1	+	+	C
GM 31875	782-18A1	+	+a	C
GM 32458	604-8A1	+	+	C
GM 32458	782-21B1	+	+	C
GM 6417-7	1347-7A1	+	+	C
GM 6497-4	1347-14A1	+	+	C
GM 7116-10	1362-4A1	+	+	C
GM 7117-13	1347-17A2	+	+	C
Grande Glabre (R)	279897	+	+a	U
Gyongyriziling	1407-4A1	+	+	C
ILL 344-1 (H)	GVIT 658	+	+a	U
Joffre (Kuhlmann 187-1) (H)	GVIT 381	+	+a	U
Koret (H)	Q1179-7	+	+b	C
Malvasia (V)	153A	+	+	I
Malvasia (V)	161A	+	+	I
Merlot CL 447 (V)	1236-17A1	+	+	C
Moureto	87	+	+	P
Moureto	96	+	+	P
Muscat De Hambourg CL 202	1346-5A1	+	+	C
Perle of Csaba	Q806-1	+	+b	C
Pinot Chardonnay CL 76 (V)	949-3A2	+	+a	C
Pinot Chardonny CL 277 (V)	949-8B1	+	+	C
Pinot Grigio (V)	104A	+	+b	I
Pinot Grigio (V)	108A	+	+b	I
Pinot Grigio (V)	114A	+	+	I
Pollux B6-18	1357-4A1	+	+	C
Pslanka (H)	23-10A2	+	+	C
Ravat 34	PI 588247	+	+a	U
Refosco (V)	190A	+	+?	I
Refosco (V)	195A	+	+	I
Riesling CL 49 (V)	1555-2A1	+	+a	C
San Giovese Brunello CL E BS 4	1497-3B1	+	+	C
Schew-Rebe	778-6A1	+	+a	C
Semillon CL 299 (V)	1555-7A1	+	+a	C
Seyval Blanc (Seyve Villard 5-276) (H)	PI 588309	+	+a	U
Seyve Villard 14-287 (H)	PI 588246	+	+a	U
Seyve Villard 3160 (H)	PI 181630	+	+a	U
Titan	Q1235-1	+	+b	C
Verdelet (H)	PI 186260	+	+a	U
Verdelho	274	+	+	P
Verduzzo (V)	222A	+	+b	I
Verduzzo (V)	226A	+	+b	I
Verduzzo (V)	239A	+	+	I

Table 5

Cultivar/Accession	ID	Index St.G	RT-PCR	Source
Vidal Blanc	1200-5A1	+	+a	C
Weiser Burgunder	Q782-40	+	+b	C
3309 C	330-4A1	+	+	C
420 A	1483-4A1	+	+	C
7542	Q1386-1	+	+b	C
Pinot Noir (V)	1186-9A2	+	+a	C
Thompson Seedless (V)	RSP105	+	+a	U

Symbols:
V., *Vitis vinefera*; R., *Vitis riparia*; H., hybrid; C., Canada; I., Italy; U., USA; P., Portugal;
a, tested by RSP149F1/R1 and 95F1/R1 and results agree to each other; b, tested by 95F1/R1 only.

Table 6

Cultivar/Accession	ID	Index St.G	RT-PCR	Source
Aligote	Q637-2B2	+	-b	C
Aragonez (Temperanillo)	232	+	-	P
Canandaigua (H)	GVIT 566	+	-a	U
Challenger (H)	Q1338-1	+	-b	C
Fercal CL 242	1551-4A1	+	-a	C
GM 7746-6	1362-6A1	+	-	C
Gravesac CL 264	1551-3A1	+	-a	C
Honey Red	1339-6A1	+	-	C
Kee-Wah-Din (H)	1278-1A1	+	-	C
Periquita	72	+	-	P
Tajoznyt Izumrud (H)	Q2-2	+	-b	C
Thurling	1047-4A2-1A2	+	-	C
Verdelet	1170-3D2-2A1	+	-	C
5BB CL 114	1236-2A1	+	-	C
Alphonse Lavalle		NI	+	I
Ancellotta		NI	+	I
Chardonnay (V)	127	NI	+	I
Kober 5BB?	100	NI	+	I
Moscato d'Adda	7	NI	+	I
Periquita	624	NI	+	P
Periquita	633	NI	+	P
Riesling (V)	3	NI	+	I
Seyval (H)	Peterson	NI	+	U
Terrano	1/1/3/K	NI	+	I
Thurling	1047-4A2-2A1	NI	-	C
Tocai Rosso 19	1586-21P4	NI	+	C
Trebbiano Toscano	67	NI	-	I
Vidal	Peterson	NI	+	U

Symbols:
V., *Vitis vinefera*; R., *Vitis riparia*; H., hybrid; NI, not indexed; C., Canada; I., Italy; U., USA; P., Portugal;
a, tested by RSP149F1/R1 and 95F1/R1 and results agree to each other; b, tested by 95F1/R1 only

Table 7

Cultivar/Accession	ID	Index St.G	RT-PCR	Source
V. acerifolia	PI 588448	NI	-	U
V. acerifolia	PI 588449	NI	-	U
V. cinerea	PI 588446	NI	-	U
V. monticola	PI 588454	NI	-	U
V. riparia	PI 495622	NI	-	U
V. sp. yenshanesis	PI 588421	NI	-	U

Symbols:
V., *Vitis vinefera*; R., *Vitis riparia*; H., hybrid; NI, not indexed; C., Canada; I., Italy; U., USA; P., Portugal;
a, tested by RSP149F1/R1 and 95F1/R1 and results agree to each other; b, tested by 95F1/R1 only

Among the 93 RSP-infected grapevines, 79 (85%) produced cDNA fragments of expected sizes in repeated RT-PCR using RSP149F1/R1 primers (SEQ. ID. Nos. 49 and 50) and/or RSP95F1/R1 primers (SEQ. ID. Nos. 47 and 48), while the other 14 were negative (see Tables 5 and 6). Interestingly, 12 of 14 (85.7%) grapevine accessions which were not indexed for RSP also produced cDNA fragments of expected size in RT-PCR (see Table 6). Sauvignon blanc (healthy control) was negative in repeated RT-PCR (see Table 3).

Results of RT-PCR for grapevines indexed negatively for RSP were surprising (see Tables 3 and 4). While 11 were negative in RT-PCR tests (excluding Sauvignon blanc healthy control), the other 13 produced cDNA fragments of expected sizes.

Since RSPaV-1 was detected not only from grapevines which indexed positively for RSP but also from some of the grapevines indexed negatively for RSP, a search for more healthy materials for RT-PCR tests became necessary. As the majority of plant viruses do not pass on through seeds, grapevine seedlings are probably free of RSPaV-1. Based on this assumption, six seedlings from five *Vitis* species were included in RT-PCR (see Table 7). None of them produce cDNA of expected size in RT-PCR using RSP149R1/F1 primers (SEQ. ID. Nos. 49 and 50).

The data described above (and shown in Tables 3-7) indicate that RSPaV-1 is closely associated with RSP and that it is likely the causal agent of RSP. RT-PCR detected RSPaV-1 specific sequences from most of the RSP-infected grapevines collected from a wide range of viticultural regions of the world. Among the 93 grapevine accessions indexed positively for RSP on St. George, 85% were positive in RT-PCR (see Table 5). The data also suggests that RT-PCR has the

potential to be used as a standard method for diagnosing RSP. This method is advantageous over the biological indexing on indicator St. George, because it is simpler, quicker, and more sensitive.

RT-PCR did not detect RSPaV-1 sequences from 14 of the grapevine accessions indexed positively for RSP (see Table 6). The discrepancy between RT-PCR and indicator indexing can be attributed to the existence in grapevines of different viruses or strains of the same virus which may all induce similar pitting and/or grooving symptoms on St. George upon graft-inoculation. It is believed these agents are only slightly different from RSPaV-1 at the level of their nucleotide sequences, but significant enough to hinder them from being detected by RT-PCR using RSPaV-1 specific primers.

It is likely that many RSPaV strains have genomes with nucleotide sequences that are highly similar to the nucleotide sequence of the RSPaV-1 genome. Evidence that supports this hypothesis includes the finding of a highly conserved region of ca. 600 bps among the nucleotide sequences of RSPaV-1 (type strain) and seven other cDNA clones, as shown in Figure 9. The nucleotide sequence identities of these strains to RSPaV-1 (type strain) range from 83.6% to 98.4%. If oligonucleotides are chosen which are conserved among all these strains (i.e., with one or only a few mismatches), then the oligonucleotides should function as universal primers, allowing all of the strains to be detected by RT-PCR. Based on this theory, a primer pair (BM98-3F/BM98-3R) can be designed to amplify a DNA fragment of 320 bps from all these clones. BM98-3F has a nucleotide sequence corresponding to SEQ. ID. No. 51 as follows:

GATGAGGTCCAGTTGTTTCC 20

BM98-3R has a nucleotide sequence corresponding to SEQ. ID. No. 52 as follows:

ATCCAAAGGACCTTTTGACC 20

Primers BM98-3F/BM98-3R can be used in RT-PCR to test further some of the grapevine samples which were negative for RSPaV in RT-PCR using RSP95F1/RSP95R1 primers (SEQ. ID. Nos. 47 and 48, respectively) or RSP149F1/RSP149R1 primers (SEQ. ID. Nos. 49 and 50, respectively). Results show that 6 of the 9 samples included were positive for RSPaV in RT-PCR using

BM98-3F/BM98-3R primers. This indicates that these universal primers can be used to achieve even higher detection rates.

Another pair of primers (BM98-1F/BM98-1R) can be designed in a way that they can amplify DNA of 760 bps from RSPaV-1, RSP47-4, and RSP158. BM98-1F has a nucleotide sequence corresponding to SEQ. ID. No. 53 as follows:

CTTGATGAGTACTTGTC 17

BM98-1R has a nucleotide sequence corresponding to SEQ. ID. No. 54 as follows:

GCAAGGATTTGGATGGC 17

Other "universal primers" can be designed manually or with computer programs (such as PrimerSelect) in the same way so that they contain conserved regions of nucleotide sequences for different strains of RSPaV-1.

RT-PCR detected RSPaV-1 sequences from 54% of grapevines negative for RSP as judged by indexing on St. George (see Tables 3 and 4). Several possibilities may account for this discrepancy. First, RT-PCR is much more sensitive than indicator indexing. Virus(es) of extremely low concentration may not induce visible symptoms on St. George within the standard indexing period, while they can be detected by RT-PCR. Second, judging indexing results can, in some cases, be very subjective. For example, it is very difficult to reach a conclusion on whether a grapevine is infected with RSP when only one or a few small pits are present on the woody cylinder of St. George. Third, uneven distribution of virus(es) within grapevines and the relatively limited number of replicates of St. George indicators may result in the failure to detect RSP-infection.

RSP seems to be widespread in different types of grapevines including *V. vinifera*, hybrids, *V. riparia*, and rootstocks. It occurs in a wide range of geographic regions including North America, Europe, Australia, and possibly many other countries as well. Testing grapevines from other areas of the world using RSPaV-1 specific primers will provide definitive information on the exact distribution of RSP throughout the world. It is also interesting to investigate whether RSP is transmitted by any vectors in nature.

RSP is a disease under quarantine in Washington and New York of the USA. Since this work and the work of others (Golino and Butler, "A Preliminary

Analysis of Grapevine Indexing Records at Davis, California," in Proceedings of the 10th Meeting of the ICVG, pp. 369-72, Rumbos et al., eds., Volos, Greece (1990); Azzam and Gonsalves, "Detection of dsRNA in Grapevines Showing Symptoms of *Rupestris* Stem Pitting Disease and the Variabilities Encountered," Plant Disease, 75:96-964 (1991); Garau, "Kober Stem Grooving and Grapevine Virus A: A Possible Relationship," in Extended Abstracts of the 11th Meeting of the International Council for the Study of Viruses and Virus Diseases of the Grapevine, p. 54, Montreux, Switzerland (1993); Credi, "Characterization of Grapevine Rugose Wood Sources from Italy," Plant Disease, 82:1288-92 (1997), all of which are hereby incorporated by reference) showed that RSP is so wide-spread, it is questionable whether or not RSP should be kept under plant quarantine any longer. The devlopment and advance of rapid diagnostic methods will also allow us to investigate on the economic damage caused by RSP.

According to Goheen ("*Rupestris* Stem Pitting," in Compendium of Grape Diseases, p. 53, Pearson and Goheen, eds., American Phytopathological Society Press, St. Paul, Minnesota, USA (1988), which is hereby incorporated by reference), RSP is a disease which induces, after graft-inoculation with a chip bud from an infected grapevine, a row of small pits on the woody cylinder of St. George below the point of inoculation. This definition may not be comprehensive. Indexing record indicated that two types of stem pitting (specific vs. nonspecific) were often observed on the woody cylinder of St. George upon graft inoculation with chip buds. For example, among 16 RSP-positive grapevines collected from Canada in 1995, eight developed specific type symptoms, while the others produced nonspecific symptoms. Credi ("Characterization of Grapevine Rugose Wood Sources from Italy," Plant Disease, 82:1288-92 (1997), which is hereby incorporated by reference) also observed these two types of stem pitting in his indexing work. However, from the primers used in RT-PCR, as described above, RSPaV-1 was detected in grapevines showing both types of symptoms on St. George.

Thus, RT-PCR detected RSPaV-1 sequences from a wide range of grapevines collected from a number of major grapevine growing countries. The data clearly suggest that RSPaV-1 is closely associated with *Rupestris* stem pitting of grapevines and that it is likely the causal virus of RSP. Use of "universal" primers which can detect multiple agents which are highly similar to RSPaV-1 in nucleotide

sequences would improve the detection rate by RT-PCR. In addition, antibodies produced against bacteria-expressed coat proteins of RSPaV-1 will help in finding the viral particles from RSP infected grapevines and in rapid detection of RSP.

Example 17 - Southern Hybridization

To confirm the specificity of the RT-PCR products to RSPaV-1, Southern blot hybridization was conducted using 32P labeled probe specific to RSPaV-1. As shown in Figure 7, the Southern blot hybridization confirmed the results of the RT-PCR in each of the tested samples. Specifically, cDNA fragments amplified by RT-PCR from 16 selected RT-PCR positive samples hybridized with the probe.

Example 18 - Constructing Expression Systems, Expression of a Fusion Protein Containing the RSPaV-1 Coat Protein, Production of Antibodies Against the Fusion Protein and Their Use in Detecting RSPaV-1 from Grapevines

The coat protein gene (SEQ. ID. No. 10) of RSPaV-1 was cloned into the EcoRI and HindIII sites of the polylinker region of a protein expression vector pMAL-c2 which, upon induction by inducer IPTG, produces a fusion protein containing maltose binding protein (MBP) and the coat protein of RSPaV-1. The fusion protein of expected size (ca. 71 KDa) was produced in *E. coli* bacteria after induction with IPTG. This fusion protein was purified through affinity chromatography using an amylose column. Purified fusion protein was used as an antigen to immunize a rabbit (by subcutaneous injection along the back) with the following scheme:

first injection, 400 µg fusion protein in 0.5 ml column buffer with Freund's complete adjuvant;

second injection, 100 µg of protein in 0.5 ml column buffer with Freund's incomplete adjuvant; and

third injection, 100 µg of protein in 0.5 ml buffer with Freund's incomplete adjuvant.

Blood containing the antibodies was collected 70 days after the first injection. The antibodies were recovered and successfully used in an enzyme linked

immunoabsorbent assay to detect the presence of virus particles (i.e., coat protein) of RSPaV-1 from a variety of tissue types of grapevines infected with RSP.

The antibodies produced against the expressed RSPaV-1 coat protein, therefore, are useful in the identification of the particles associated with RSP disease of grapevines, in the purification of the particles of RSPaV-1, and in the development of a serological diagnosis for RSP in grapevine. The use of the antibodies is suitable for detecting different strains of RSPaV-1. Because the coat proteins for strains RSP47-4 and RSP158 have high amino acid identities with the coat protein of RSPaV-1, it is very likely that the antibodies raised against RSPaV-1 coat protein will also detect other strains. Antibodies can be used in an ELISA to assay rapidly a large number of samples, thus making commercial development and utilization of diagnostic kits possible.

Example –19 Transformation of Grapevines with a Vector Containing RSPaV-1 Coat Protein Gene and Analysis of Transgenic Grapevines for Resistance to RSP

The DNA molecule coding for the RSPaV-1 coat protein (e.g., SEQ. ID. No. 10) was cloned into a pEPT8 plant expression vector that contains the double 35S enhancer at restriction sites SalI and BamHI. The resulting recombinant plasmid, designated pEPT8/RSPaV-1 coat protein, was then cloned into the plant transformation vector pGA482G, which has resistance genes to gentamycin and tetracycline as selection markers. The resultant pGA482G containing pEPT8/RSPaV-1CP was used to transform grapevines using the *Agrobacterium* method.

The rootstock *Vitis rupestris* Scheele St. George was used in genetic transformation. Anthers were excised aseptically from flower buds. The pollen was crushed on a microscope slide with acetocarmine to observe the cytological stage (Bouquet et al., "Influence du Gentype sur la Production de cals: Dembryoides et Plantes Entieres par Culture Danthers in vitro dans le Genre Vitis," C.R. Acad. Sci. Paris III 295:560-74 (1982), which is hereby incorporated by reference). This was done to determine which stage was most favorable for callus induction.

Anthers were plated under aseptic condition at a density of 40 to 50 per 9 cm diameter Petri dish containing MSE. Plates were cultured at 28°C in the dark. After 60 days, embryos were induced and transferred to hormone-free medium

(HMG) for differentiation. Torpedo stage embryos were transferred to MGC medium to promote embryo germination. Cultures were maintained in the dark at 26-28°C and transferred to fresh medium at 3-4 week intervals. Elongated embryos were transferred to rooting medium (5-8 embryos per jar). The embryos were grown in a tissue culture room at 25°C with a daily 16 h photoperiod (76 μmol. s) to induce shoot and root formation. After plants developed roots, they were transplanted to soil in the greenhouse.

The protocols used for transformation were modified from those described by Scorza et al., "Transformation of Grape (*Vitis vinifera L.*) Zygotic-Derived Somatic Embryos and Regeneration of Transgenic Plants," Plant Cell Rpt. 14:589-92 (1995), which is hereby incorporated by reference. Overnight cultures of *Agrobacterium* strain C58Z707 or LBA4404 were grown in LB medium at 28°C in a shaking incubator. Bacteria were centrifuged for 5 minutes at 3000-5000 rpm and re-suspended in MS liquid medium (OD 1.0 at A600 nm). Calli with embryos were immersed in the bacterial suspension for 15-30 minutes, blotted dry, and transferred to HMG medium with or without acetosyringone (100 μM). Embryogenic calli were co-cultivated with the bacteria for 48 h in the dark at 28°C. The plant material was then washed in MS liquid plus cefotaxime (300 mg/ml) and carbenicillin (200 mg/ml) 2-3 times. To select transgenic embryos, the material was transferred to HMG medium containing either 20 or 40 mg/L kanamycin, 300 mg/L cefotaxime, and 200 mg/L carbenicillin. Alternatively, after co-cultivation, embryogenic calli were transferred to initiation MSE medium containing 25 mg/l kanamycin plus the same antibiotics listed above. All plant materials were incubated in continuous darkness at 28°C. After growth on selection medium for 3 months, embryos were transferred to HMG or MGC without kanamycin to promote elongation of embryos. They were then transferred to rooting medium without antibiotics. Non-transformed calli were grown on the same media with and without kanamycin to verify the efficiency of the kanamycin selection process.

The X-gluc (5-bromo-4-chloro-3-indoyl-ß-glucuronidase) histochemical assay was used to detect GUS (ß-glucuronidase) activity in embryos and plants that were transformed with constructs containing the GUS gene that survived kanamycin selection. All propagated plants were screened using an enzyme linked immunoabsorbent assay (ELISA) system (5 Prime-3 Prime, Boulder, Co.) to

detect the NPTII (neomycin phosphotransferase II) protein in leaf extracts. ELISA tests with respective coat protein (CP)-specific antibodies were used to assay for CP. ELISA results were read on an SLT Spectra ELISA reader (Tecan U.S. Inc., Research Triangle Park, NC) 15-60 minutes after the substrate was added.

PCR analysis was carried out to detect the presence of transgene sequences in grape plants. Genomic DNA was isolated from transformed and non-transformed grape plants according to the method of Lodhi et al., "A Simple and Efficient Method for DNA Extraction from Grapevine Cultivars and Vitis Species," Plant Mol. Biol. Rpt. 12:6-13 (1994), which is hereby incorporated by reference. Primer sets included those of specific primers to the transgene. DNA was initially denatured at 94°C for 3 minutes, then amplified by 35 cycles of 1 minute at 94°C (denaturing), 1 minute at 52°C (annealing), and 2 minutes at 72°C (polymerizing). Reaction samples were directly loaded and electrophoresed in 1.5 % agarose gels.

Southern analysis of transformants was accomplished by extracting genomic DNA from young leaves of transformed and non-transformed plants (3309C) as described above. DNA (10 μg) was digested with the restriction enzyme *Bgl* II, electrophoresed on a 0.8% agarose gel in TAE buffer and transferred to a Genescreen Plus membrane by capillary in 10 x SSC. A probe was prepared by random primer labeling of a PCR amplified gene coding sequence with radioisotope ^{32}P-dATP (Dupont, NEN). Pre-hybridization and hybridization steps were carried out at 65°C following the manufacturer's instruction. The autoradiograph was developed after overnight exposure.

Although the invention has been described in detail for the purposes of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.